Quality Assurance Project Plan for

Fish Plug Evaluation Study Sample Collection and Preparation

Revision 2
July 19, 2018

Prepared for:

United States Environmental Protection Agency
Office of Water
Office of Science and Technology
Standards and Health Protection Division

Prepared with support from:

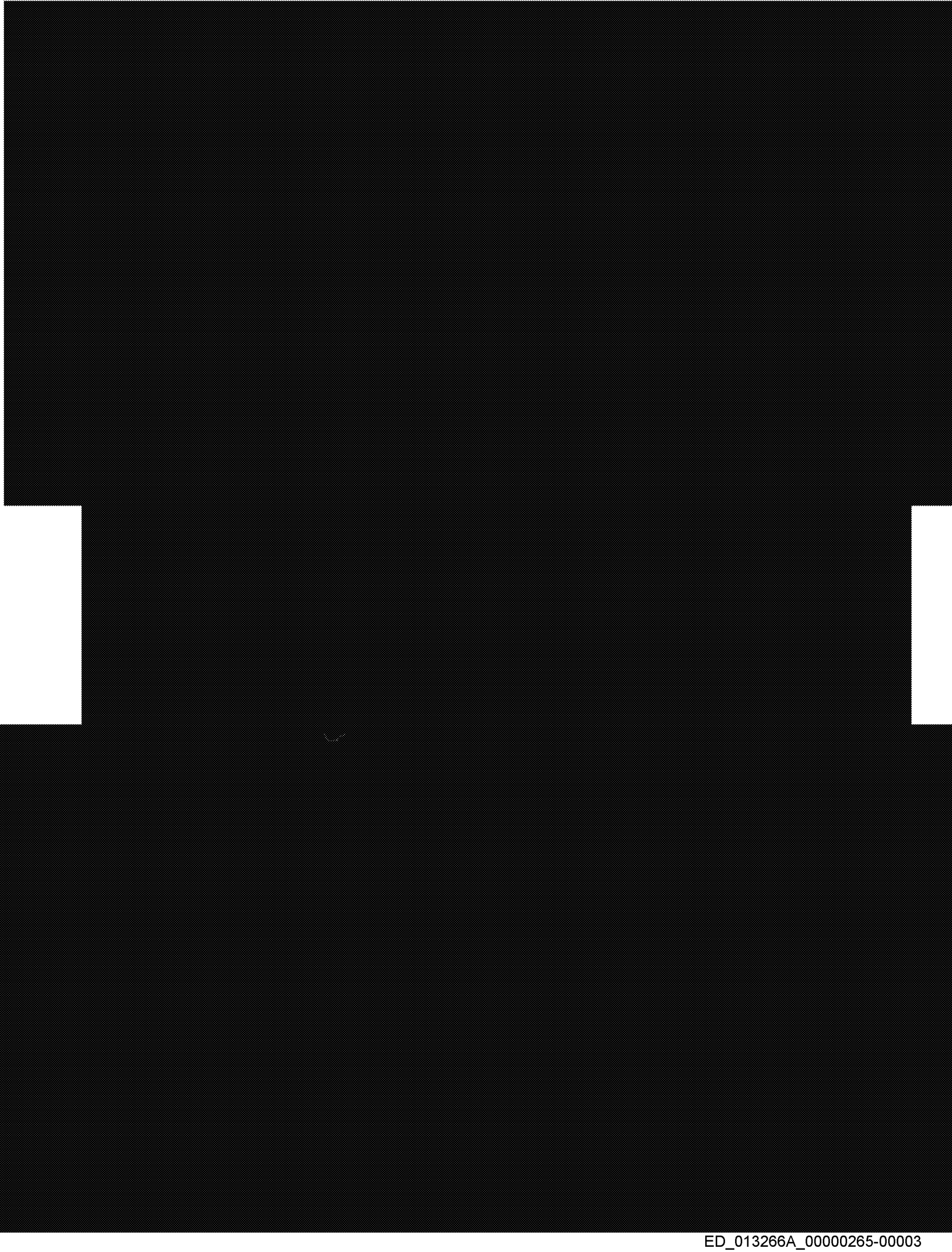
Tetra Tech, Inc.
under:
Standards and Health Protection Division
[redacted]

Quality Assurance Project Plan for Fish Plug Evaluation Study Sample Collection and Preparation

A. PROJECT MANAGEMENT

The initial Quality Assurance Project Plan (QAPP) for the Fish Plug Evaluation Study presented study objectives, the design and phased implementation plan, and the procedures for collecting, handling, and shipping whole fish samples and field-extracted fillet plug samples to the laboratory designated for fillet tissue sample preparation. Revision 1 of the initial QAPP added procedures for preparation of fillet tissue samples (homogenized whole fillet tissue samples and lab-extracted plug samples) for mercury analysis and procedures and requirements for lipid analysis of homogenized whole fillet tissue samples from all fish (90) collected for the study (60 fish for the mercury phase and 30 fish for the selenium phase as shown in Figure 2 and Figure 3, respectively). This revision (Revision 2) incorporates changes to the selenium phase of the plug study and adds procedures for preparation of homogenized whole fillet tissue samples for selenium analysis. A QAPP describing methods and requirements for analysis of Fish Plug Evaluation Study fillet tissue samples for mercury was developed by CSRA under a separate EPA contract ([redacted]) on September 29, 2017 (USEPA 2017), and it was revised to add analyses of fillet tissue samples for selenium and percent solids on June 6, 2018 (USEPA 2018).

The U.S. Environmental Protection Agency's (EPA's) Office of Science and Technology (OST) within the Office of Water (OW) prepared this QAPP Revision 2 with support from Tetra Tech under EPA Contract No. [redacted]. It was prepared in accordance with the most recent version of EPA QA/R-5, *EPA Requirements for Quality Assurance Project Plans* (USEPA 2001), that was reissued in 2006. This QAPP is a dynamic document that has changed as the Fish Plug Evaluation Study moves into the selenium analysis phase. Changes to procedures in this QAPP must be reviewed by the EPA Project Manager for the Fish Plug Evaluation Study and the EPA Standards and Health Protection Division (SHPD) Quality Assurance Coordinator to determine whether the changes will impact the technical and quality objectives of the project. If so, the QAPP is revised accordingly, circulated for approval, and forwarded to all project participants listed in the QAPP distribution list (Section A3). Key project personnel and their roles and responsibilities are discussed in the QAPP section to follow (Section A4), and information on project background and description is provided in Sections A5 and A6, respectively.



A2. Table of Contents

TABLES

FIGURES

APPENDICES

LIST OF ACRONYMS AND ABBREVIATIONS

COB	Close of business
CVAA	Cold-vapor atomic absorption
DI	Deionized
DQOs	Data Quality Objectives
EPA	Environmental Protection Agency
FPES	Fish Plug Evaluation Study
g	grams
GPS	Global positioning system
HDPE	High-density polyethylene
ICP/MS	Inductively coupled plasma-mass spectrometer
ID	Identification
LCS	Laboratory control sample
MDL	Method detection limit
mm	millimeters
OST	Office of Science and Technology
OW	Office of Water
OWOW	Office of Wetlands, Oceans, and Watersheds
QA	Quality assurance
QAPP	Quality Assurance Project Plan
QC	Quality control
RSD	Relative standard deviation
SD	Standard deviation
SHPD	Standards and Health Protection Division
SOP	Standard Operating Procedure
TBD	To be determined
WQC	Water quality criterion

A3. Distribution List

A4. Project/Task Organization

The project team for Fish Plug Evaluation Study fish sample collection and preparation consists of the EPA Project Manager in OST/SHPD, the EPA QA Officer in OST, the EPA QA Coordinator in OST/SHPD, the Tetra Tech Project Leader, and Tetra Tech staff providing scientific, technical, and logistical support for the study. The project team organization provides

the framework for conducting fish sample collection and preparation to meet study objectives. The organizational structure and function also facilitate project performance and adherence to quality control (QC) procedures and quality assurance (QA) requirements. The project organizational chart is presented in Figure 1. It identifies individuals serving in key roles and the relationships and lines of communication among these project team members. Responsibilities for key members of the project team are described below.

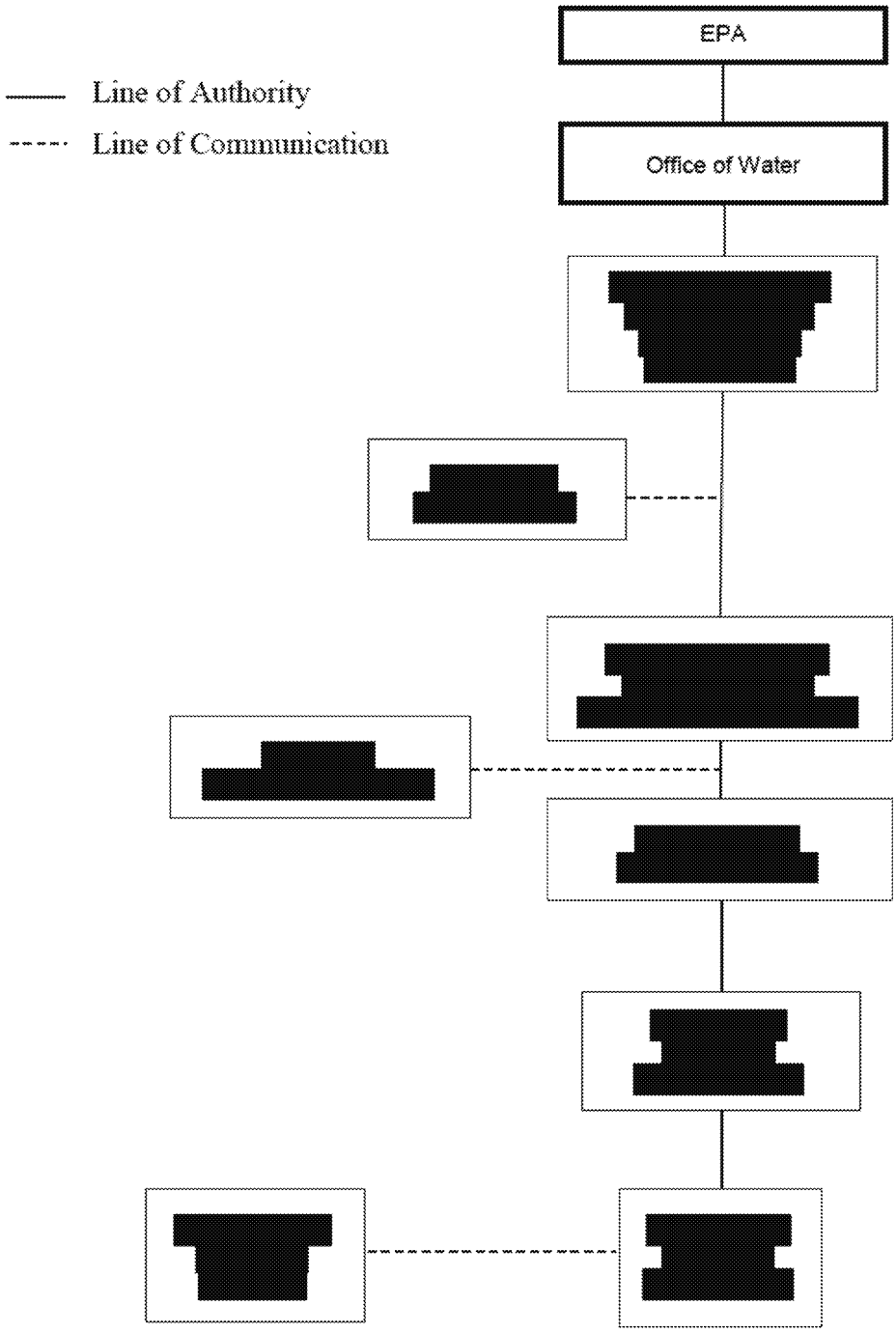


Figure 1. Fish Plug Evaluation Study (FPES) Project Team Organization for Fish Sample Collection and Preparation

[redacted] **EPA Project Manager** who is providing overall direction for planning and implementing the Fish Plug Evaluation Study. This role involves the following responsibilities related to this study:

- coordinating with project team members to plan the study design
- directing development of this QAPP for fish sample collection and preparation, and any future QAPP revisions
- reviewing and approving the Fish Plug Evaluation Study sample collection procedures and related field sampling materials, including training materials
- providing technical oversight for developing and implementing the fish sample preparation procedures and technical support for reviewing fish sample preparation QA/QC data
- managing analysis of fish fillet samples (plug and homogenized fillet samples) for target chemicals (mercury and selenium), which includes obtaining technical support for chemical analysis of fillet tissue samples, directing development of a sample analysis QAPP, providing for QA/QC review of the analytical results, developing the data files for statistical analysis of the data, reviewing and approving the final analytical QA report, and providing oversight for development of the database to store Fish Plug Evaluation Study plug and homogenized fillet results
- facilitating communication among project team members and coordinating with all of these individuals to ensure technical quality and adherence to QA/QC requirements
- developing and managing work assignments under OST or other EPA contracts to provide technical and logistical support for the Fish Plug Evaluation Study, providing oversight of all contractor activities, and reviewing and approving study deliverables for each work assignment
- scheduling and leading meetings and conference calls with project team members for planning study activities, reporting progress on study tasks, and discussing and resolving technical or other issues related to the study
- working with QA staff to identify corrective actions necessary to ensure that study quality objectives are met
- managing the development of and/or reviewing and approving all major work products associated with the Fish Plug Evaluation Study
- leading the process for planning and conducting statistical analysis of the Fish Plug Evaluation Study data
- collaborating with the project team for reporting the study results in technical journal articles and federal technical reports
- preparing presentations related to the Fish Plug Evaluation Study and presenting them in various forums (e.g., scientific conferences, government meetings, and webinars)

[redacted] **OST Quality Assurance Officer** who is responsible for reviewing and approving all Quality Assurance Project Plans (QAPPs) that involve scientific work being conducted by OST. [redacted] **Standards and Health Protection Division QA Coordinator** who is responsible for reviewing and recommending approval of all QAPPs that include scientific work being conducted by the Standards and Health Protection Division (SHPD) within OST. The OST QA Officer and SHPD QA Coordinator are also responsible for the following QA/QC activities:

- reviewing and approving this QAPP
- reviewing and evaluating the QA/QC requirements and data for all the Fish Plug Evaluation Study activities and procedures
- conducting external performance and system audits of the procedures applied for all Fish Plug Evaluation Study activities
- participating in Agency QA reviews of the study

[redacted] **Tetra Tech Project Leader** who is responsible for managing all aspects of the technical and logistical support being provided by Tetra Tech staff for the Fish Plug Evaluation Study. His specific responsibilities include the following:

- providing direct technical and logistical support for the following Fish Plug Evaluation Study activities or providing leadership and oversight for Tetra Tech staff supporting these activities:
  - developing procedures for fish sampling and fish sample preparation
  - preparing plug study documents (including this QAPP)
  - providing fish sampling and fish sample preparation training
  - planning and implementing Fish Plug Evaluation Study logistics
  - conducting fish sampling at Great Lake and mid-Atlantic river sites designated by the EPA Project Manager
  - recording Fish Plug Evaluation Study fish sampling data and assigning staff to perform independent QA/QC reviews of these data
  - assigning batches for fish sample preparation and managing implementation of the fish sample preparation procedures
  - preparing weekly fish processing reports and evaluation the reports for adherence to the technical and quality requirements in the fish sample preparation procedures
  - packing and shipping fish tissue samples to analytical laboratories designated for mercury and selenium analyses
  - preparing project information and graphics for development of project fact sheets, presentations, and other EPA meeting and outreach materials
  - providing technical support for planning and conducting statistical analysis of Fish Plug Evaluation Study data and reporting the final results

- monitoring the performance of Tetra Tech staff participating in this study to ensure that they are following all QA procedures described in this QAPP that are related to Tetra Tech tasks being performed to support this study
- ensuring completion of high-quality deliverables within established budgets and time schedules
- participating in meetings and conference calls with project team members for planning study activities, reporting progress on study tasks, and discussing and resolving technical issues related to the study

[redacted] **Tetra Tech QA Officer** whose primary responsibilities include the following:

- assisting Tetra Tech's Project Leader with the development and review of this QAPP
- approving this QAPP
- providing oversight for the implementation of QA procedures related to Tetra Tech tasks that are described in this QAPP
- reporting deviations from this QAPP to the Tetra Tech Project Leader and assisting in implementing corrective actions to resolve these deviations

A5. Problem Definition/Background

In 2013, EPA's Office of Wetlands, Oceans, and Watersheds (OWOW) within OW initiated fish plug sampling from whole fish collected at 361 river sites for the 2013-14 National Rivers and Streams Assessment (NRSA) human health fish tissue indicator. Collecting and analyzing fillet plug samples was applied as a more cost-effective alternative to obtain mercury data for human health applications than the routine approach of removing entire fillets from each fish in a sample and homogenizing the fillet tissue for mercury analysis. OWOW expanded use of fish fillet plug sampling for mercury analysis during the 2015 National Coastal Condition Assessment (NCCA) by applying this fish tissue sampling technique on fish samples collected from the 225 Great Lakes sites and the 684 marine sites along the coasts of the contiguous United States that were designated for fish sampling.

Prior to these EPA surveys, a few states were experimenting with fish plug sampling to monitor mercury contamination in fish. EPA's widespread use of fish plug sampling in these two recent National Aquatic Resource Surveys has prompted more states to introduce this sampling technique into their fish monitoring programs. However, the question remains about whether fish fillet plug sampling and analysis can serve as a reliable surrogate for the traditional approach of homogenizing and analyzing whole fillet tissue to monitor mercury concentrations in fish. Additionally, this study will investigate if it is technically feasible to apply fillet plug sampling and analysis to monitor selenium concentrations in fish.

A6. Project/Task Description

OST is conducting the Fish Plug Evaluation Study to address the fundamental question about comparability of fillet concentration results when analyzing fish fillet plug samples vs.

homogenized whole fillet tissue samples for mercury and selenium. Data from this study should allow EPA to determine if fish fillet plug sampling and analysis can be applied as a technically comparable alternative to homogenizing and analyzing whole fillet tissue samples for these two metals. Depending on the outcome of the study, there could be important cost implications if study data demonstrate that fish fillet plug analysis can serve as a reliable alternative for monitoring levels of mercury and selenium in fish. A positive outcome for the study would be to identify fish fillet plug sampling and analysis as an effective approach that state and federal agencies can use for surveillance and compliance monitoring of mercury and selenium levels in fish at much lower costs.

The design of the Fish Plug Evaluation Study allows for conducting the study in two phases: the mercury phase and the selenium phase. EPA began implementing the mercury phase of the study in June 2017 and initiated the selenium phase in May 2018. Fish sampling for the Fish Plug Evaluation Study is being conducted in two waterbody types: the Great Lakes and mid-Atlantic rivers. Three target species consisting of lake trout, walleye, and Chinook salmon are being collected from three Great Lakes. Three additional target species (i.e., largemouth bass, smallmouth bass, and blue catfish) are being collected from three mid-Atlantic rivers (refer to Section B1 for more study design details).

Mercury Phase

The mercury phase involves collecting and analyzing a greater number of fillet tissue samples (900 samples) to thoroughly test the comparability of results from analysis of the following three types of fillet samples for mercury: field-extracted fillet plug samples, lab-extracted fillet plug samples, and homogenized whole fillet tissue samples. Ten individual fish of each of the six target species (a total of 60 fish) and five field-extracted fillet plug samples from each whole fish sample (a total of 300 plug samples) were collected for the mercury phase of the study during August and September 2017. The remaining 600 fillet samples for the mercury phase of the study consist of 300 lab-extracted fillet plug samples and 300 homogenized whole fillet samples (five replicates per whole fish for each type of fillet sample).

Selenium Phase

The selenium phase of the study builds on the results from the mercury phase and involves analysis of a smaller number (360 samples) of the same three types of fillet samples for selenium: field-extracted fillet plug samples, lab-extracted fillet plug samples, and homogenized whole fillet tissue samples. Five individual fish of each of the six target species (a total of 30 fish) and four field-extracted fillet plug samples from each whole fish sample (a total of 120 plug samples) are being collected for the selenium phase of the study. The remaining 240 fillet samples for the selenium phase of the study consist of 120 lab-extracted fillet plug samples and 120 homogenized whole fillet samples (four replicates per whole fish for each type of fillet sample). The selenium phase also provides the opportunity to determine if using small tissue volumes is technically feasible to monitor selenium levels in fish below the WQC of 11,300 ng/g on a dry-weight basis.

Note: In contrast to mercury, which can be measured at very low levels (sub parts per billion) in fish tissue, readily available analytical methods for selenium in fish tissue are much less

sensitive and sample size will be a limiting factor for this study. Therefore, the focus of the selenium phase is not on "how low you can go," but whether or not measurements can be made at or below the WQC using fillet plug samples.

A7. Quality Objectives and Criteria

Data of known and documented quality are essential to the success of any sampling program. Data quality objectives (DQOs) are qualitative and quantitative statements that clarify the intended use of the data, define the type of data needed to support the decision, identify the conditions under which the data should be collected, and specify tolerable limits on the probability of making a decision error due to uncertainty in the data. DQOs are developed by data users to specify the data quality needed to support specific decisions. Sources of error or uncertainty include the following:

- Sampling error: The difference between samples values and *in situ* true values from unknown biases due to collection methods and sampling design.
- Measurement error: The difference between sample values and in situ true values associated with the measurement process.
- Natural variation: Natural spatial heterogeneity and temporal variability in population abundance and distribution.
- Error sources or biases associated with compositing, sample handling, storage, and preservation.

This QAPP addresses activities associated with fish sample collection and preparation, so the relevant quality objectives are related to issues involving sample collection and handling in the field and fillet tissue sample preparation in the laboratory. Table 1 lists the types of fish sampling and tissue sample preparation data needed for the Fish Plug Evaluation Study. Methods and procedures described in this document are intended to reduce the magnitude of the sources of uncertainty and their frequency of occurrence by applying the following approaches:

- Use of standardized sample collection, handling, and preparation procedures
- Use of trained scientists to perform the sample collection, handling, and preparation activities

Table 1. Types of Field and Laboratory Data to Be Collected in Association with Fish Sample Collection and Preparation for the Fish Plug Evaluation Study

Data Type	Measurement Endpoint(s) or Units
Fish specimen (field)	Species-level taxonomic identification
Fish length (field)	Millimeters (mm), total length
Fish weight (laboratory)	Grams (g)
Unhomogenized fillet weight (laboratory)	Grams (g)
Homogenized fillet weight (laboratory)	Grams (g)
Plug weight (laboratory)	Grams (g)
Tissue homogenate recovery (laboratory)	Percent
Tissue aliquot weight (laboratory)	Grams (g)
Tissue archive weight (laboratory)	Grams (g)

Measurement performance criteria are quantitative statistics that are used to interpret the degree of acceptability or utility of the data to the user. These criteria, also known as data quality indicators, include the following:

- Precision
- Accuracy
- Representativeness
- Completeness
- Comparability

Precision

Precision is a measure of internal method consistency. It is demonstrated by the degree of agreement between individual measurements (or values) of the same property of a sample measured under similar conditions. The only analytical testing that is within the scope of this QAPP is the analysis of fish preparation rinsate samples for mercury and selenium (to ensure that the preparation laboratory environment and equipment are not an extraneous source of mercury or selenium) and lipid analysis to test the homogeneity of the prepared fish fillet tissue samples and to provide lipid results for the full complement of 90 fish collected during the study (60 fish for the mercury phase and 30 fish for the selenium phase). Sufficient fillet tissue sample replicates will be prepared to allow for the assessment of precision during full analytical laboratory testing for the Fish Plug Evaluation Study, as described in the sample analysis QAPP (USEPA 2018).

For the mercury phase of the plug study, the sample preparation laboratory will prepare one set of rinsate samples (consisting of one deionized [DI] water equipment rinsate sample and one DI water blank sample) for mercury analysis and one fillet sample for triplicate lipid determinations per fish sample preparation batch containing two fish samples, as described in Steps 31 through 36 of Appendix B. The two fish samples in each fish sample preparation batch generate a mercury analysis batch that includes 10 lab-extracted plug samples and 10 homogenized fillet tissue samples (five replicates of each type of tissue sample from each of two fish as described in

Section B1). The batch-specific rinsate and homogeneity results are reviewed by EPA against the QC specifications detailed in Section B5.2.

For the selenium phase of the plug study, the sample preparation laboratory will prepare one set of rinsate samples (consisting of one deionized [DI] water equipment rinsate sample and one DI water blank sample) for selenium analysis and one fillet sample for triplicate lipid determinations per fish sample preparation batch containing five fish samples, as described in Steps 32 through 37 of Appendix D. The five fish samples in each fish sample preparation batch generate two selenium analysis batches that include 20 lab-extracted plug samples and 20 homogenized fillet tissue samples (four replicates of each type of tissue sample from each of five fish as described in Section B1). The batch-specific rinsate and homogeneity results are reviewed by EPA against the QC specifications detailed in Section B5.2.

Accuracy

Accuracy is defined as the degree of agreement between an observed value and an accepted reference or true value. Accuracy is a combination of random error (precision) and systematic error (bias) introduced during sampling and analytical operations. Bias is the systematic distortion of a measurement process that causes errors in one direction, so that the expected sample measurement is always greater or lesser to the same degree than the sample's true value. Proper sample handling procedures will be followed to minimize sample contamination during sample collection and shipment (Section B2), sample preparation (Section B4), and QC analysis (Section B5.2).

Representativeness

Representativeness expresses the degree to which data accurately and precisely represent a characteristic of a population, a parameter, a process condition, an environmental condition, or variations at a sampling point. The representativeness goal for the Fish Plug Evaluation Study sample collection effort will be satisfied by using experienced field biologists to ensure that the samples collected are actually of the type (species) specified for this study and are from the target waterbodies. The Field Team Leader, who must be an experienced fisheries biologist, will assess conditions in the field and determine the most appropriate locations within each target waterbody to collect the fish. The sampling site coordinates (latitude and longitude) and a brief description of the site where each individual fish is collected will be recorded on the Sample Collection Form (Appendix A).

Completeness

Completeness is defined as the percentage of measurements made that are judged to be valid according to specific criteria and entered into the data management system. To optimize completeness, every effort is made to avoid sample and/or data loss. Accidents during sample transport or lab activities that cause the loss of the original samples will result in irreparable loss of data, which will reduce the ability to perform analyses, integrate results, and prepare reports. Whole fish samples and fillet plug samples collected in the field will be packed in unbreakable (plastic) containers (i.e., insulated ice chests) to avoid damage during transport or shipment of

the samples to the fish sample preparation laboratory. Fillet homogenates and lab plug samples will also be packed in unbreakable containers for shipment to the analytical laboratory.

Percent completeness (%C) for measurement parameters can be defined as follows:

$$\%C = \frac{v}{T} \times 100$$

Where v = the number of measurements judged valid and
T = the total number of measurements.

Completeness for the Fish Plug Evaluation Study sample collection and preparation efforts is the number of valid samples collected and processed relative to the number of samples that are planned to be collected and analyzed. The completeness goal for this study is 100% because collecting and processing the target number of samples is critical for maintaining the integrity of the statistical design for the study. It should be noted that sampling locations may change over the course of the study based on location conditions (e.g., accessibility of target locations) and the availability of target species (e.g., natural biological abundance or distribution). Any changes must be approved by the EPA Project Manager, and approved changes must be considered when assessing completeness. The completeness goal is achieved when the following requirements are met:

- 60 whole fish and 300 field-extracted fillet plug samples are collected for the mercury phase of the study
- 30 whole fish and 120 pairs of field-extracted double-plug (for selenium analysis) and single-plug (for percent solids analysis) fillet samples are collected for the selenium phase of the study
- All whole fish and field-extracted fillet plug samples are shipped with no errors in documentation or sample handling procedures, which results in timely delivery of every shipment of samples and arrival of the samples at the fish sample preparation laboratory in good condition.
- 300 lab-extracted fillet plug samples and 300 homogenized fillet samples are prepared in the laboratory for the mercury phase of the study
- 120 pairs of double-plug (for selenium analysis) and single-plug (for percent solids analysis) lab-extracted fillet plug samples and 120 homogenized fillet samples with sufficient tissue mass for selenium and percent solids analyses are prepared in the laboratory for the selenium phase of the study
- Five replicates each of three types of fish tissue samples yielding 900 total fish fillet tissue samples (60 fish x 3 fillet sample types per fish x 5 replicates per fillet sample type = 900 fish fillet tissue samples) are shipped for mercury analysis with no errors in

documentation or sample handling procedures, which results in timely delivery of every shipment of samples and arrival of the samples at the analytical laboratory in good condition.

- Four replicates each of three types of fish fillet samples yielding 360 pairs of fish fillet tissue samples (30 fish x 3 fillet sample types per fish x 4 replicates per fillet sample type = 360 pairs of fish fillet tissue samples) are shipped for selenium and percent solids analyses with no errors in documentation or sample handling procedures, which results in timely delivery of every shipment of samples and arrival of the samples at the analytical laboratory in good condition.

Comparability

Comparability is an expression of the confidence with which one data set can be compared with another. Comparability is dependent on the proper design of the sampling program and on adherence to accepted sampling techniques, procedures, and quality assurance guidelines. For fish sample collection and preparation, comparability of data is accomplished by standardizing the sampling season, the field sampling methods, the field training, the sample preparation methods, and the laboratory training as follows:

- All samples for the mercury phase of the study were collected during the summer and fall of 2017 (August – October), the Great Lakes samples for the selenium phase of the study were collected during June 2018, and the rivers samples for the selenium phase of the study are scheduled for collection in July 2018. Adjustments to the river fish collection schedules during the selenium phase may be necessary due to weather, water, or other conditions. All schedule adjustments must be approved by the EPA Project Manager.

- All samples are collected and prepared for shipment according to sample collection procedures contained in this QAPP (Appendix A).

- All lab-extracted plug and homogenized fillet samples are prepared by sample preparation laboratory personnel according to the procedures contained in this QAPP (Appendix B for the mercury phase and Appendix D for the selenium phase).

- All field and laboratory personnel involved with fish sample collection and tissue sample preparation will have adequate training and appropriate fish sampling and sample preparation experience (Section A8).

A8. Special Training/Certification

All field personnel involved in the collection of whole fish and field-extracted fillet plug samples must be proficient in performing all field activities as required by the Fish Plug Evaluation Study Sample Collection Procedures in Appendix A. Each field sampling team is required to have both knowledge and experience in the collection and identification of target fish species, in the use of fisheries sampling gear specified for the study, and in the safe operation of small boats. To meet

these requirements, field sampling teams are composed of Tetra Tech biologists with strong technical backgrounds in fish sampling activities.

Specialized training was provided for all field personnel who collected, handled and shipped whole fish and fillet plug samples for the mercury phase of the Fish Plug Evaluation Study. This training was conducted at the Tetra Tech Biological Research Facility in Owings Mills, MD to accomplish the following objectives:

- summarize the Fish Plug Evaluation Study sampling design and objectives
- present sample collection and handling procedures developed for the study
- familiarize field personnel with the study-specific forms and labels used to document sample collection efforts
- review the fish and fillet plug preservation and shipping requirements, including the minimum amounts of dry ice for each type of sample preservation and shipment, shipping documentation that must accompany each type of sample shipment, the level of service required for shipping the samples (i.e., priority overnight air delivery service), and the requirements for tracking shipments and notifying the EPA Project Manager about time of sample shipment delivery and sample condition upon arrival at the fish sample preparation laboratory.

Refresher training will be provided in 2018 for all field personnel assigned to collect, handle, and ship whole fish and fillet plug samples for the selenium phase of the Fish Plug Evaluation Study.

All laboratory staff involved in the preparation of fish fillet tissue samples must be proficient in the associated tasks, as required by the Fish Plug Evaluation Study Mercury Phase Fillet Tissue Sample Preparation and Distribution Procedures (Appendix B) and by the Fish Plug Evaluation Study Selenium Phase Fillet Tissue Sample Preparation and Distribution Procedures (Appendix D). Specialized training is being provided for laboratory technicians who will be preparing fillet tissue samples, including lab-extracted plug samples and homogenized fillet tissue samples for this project. This training will be conducted at the Tetra Tech Biological Research Facility in Owings Mills, MD for all laboratory staff involved with Fish Plug Evaluation Study fillet tissue sample preparation to accomplish the following objectives:

- Present laboratory plug extraction procedures and homogenized fillet tissue sample preparation and distribution procedures for both types of fillet samples as described in Appendix B and Appendix D,
- Demonstrate laboratory plug sample collection techniques with practice fish provided by the sample preparation laboratory,
- Demonstrate filleting and homogenizing techniques with practice fish provided by the sample preparation laboratory,

- Provide hands-on opportunities for fillet tissue sample preparation laboratory staff to develop proficiency with plug sample extraction and with filleting and homogenizing fish fillet samples, including equipment cleaning procedures and production of equipment rinsate samples.

A9. Documents and Records

Thorough documentation of all Fish Plug Evaluation Study fish sample collection, handling, and processing activities is necessary for proper sample processing in the laboratory and, ultimately, for the interpretation of study results. Fish sample collection and handling will be documented in writing (for each sampling site) using the following forms and labels:

- A Sample Collection Form that contains information about each individual whole fish sample and the associated five field-extracted fillet plug samples that are collected for the mercury phase of the study (Appendix A, Exhibit 3), or one that contains information about each individual whole fish sample and the associated four field-extracted fillet plug samples and the four percent solids (identified as percent moisture for reporting data purposes) plug samples that are collected for the selenium phase of the study (Appendix A, Exhibit 4).

- A Field Plug Label that accompanies and identifies each fillet plug sample extracted in the field for the mercury phase (Appendix A, Exhibit 5), or labels that accompany and identify each fillet plug sample (Appendix A, Exhibit 6) and percent solids (identified as percent moisture for data reporting purposes) plug sample (Appendix A, Exhibit 7) extracted in the field for the selenium phase.

- A Whole Fish Label that accompanies and identifies each fish sample (Appendix A, Exhibit 8).

- A Chain-of-Custody Form that accompanies each shipment to the sample preparation laboratory and identifies each sample in every cooler (Appendix A, Exhibit 9).

- A Chain-of-Custody Label that seals each sample cooler shipped via priority overnight air delivery service (Appendix A, Exhibit 10).

A detailed description of each sample collected by a field sampling team is recorded on a Sample Collection Form. This form documents the sampling date and time, sampler's name(s), sample collection method, sampling site location and description (including latitude and longitude), and sample description (species name, fish length). The Sample Collection Form also contains a unique sample identification number that is used to identify each whole fish and fillet plug sample. The eight-character sample identification number consists of the following codes and abbreviations:

- Waterbody type (two-character abbreviation of GL=lake or RV=river)

- Site identifier (two-character abbreviation for each of the three Great Lakes and three mid-Atlantic rivers) (see the Sample Collection Form site abbreviations in Appendix A, Exhibit 3 and Exhibit 4)

- Species abbreviation (two-character abbreviation for each of the six target fish species) (see the Sample Collection Form species abbreviations in Appendix A, Exhibit 3 and Exhibit 4)

- Whole fish specimen number (i.e., a two-digit number from 01 to 10 for the mercury phase and from 01-05 for the selenium phase)

The Sample Collection Form (Appendix A, Exhibit 3 and Exhibit 4) is produced as a one-page hard copy form on waterproof paper. All entries on the form are made in ink and no erasures are made. If an incorrect entry is made, the information is crossed out with a single strike mark, which is initialed and dated by the sampler/recorder.

The Field Plug Labels (Appendix A, Exhibits 5, 6, and 7) are completed to accompany each field fillet plug sample throughout the process of transporting or shipping them to the sample preparation laboratory in Owings Mills, MD. For the mercury phase, this involves completing the label displayed in Exhibit 5. For the selenium phase, this involves labeling the double-plug field fillet sample (Exhibit 6) for selenium analysis and the single-plug fillet sample for percent solids (identified as percent moisture for data reporting purposes) analysis (Exhibit 7). The labels document the project name, sampling location, sampling date and time, 11-character sample identification number for metals analyses and 13-character sample identification number for percent solids analysis (marked and reported as percent moisture analysis), and fish species and length. All entries are made in indelible ink, and they coincide with field sampling and fillet plug sample information on the Sample Collection Form.

A Whole Fish Label (Appendix A, Exhibit 8) is completed to accompany each fish sample throughout the process of transporting or shipping fish samples to the sample preparation laboratory in Owings Mills, MD. The label documents the project name, sampling location, sampling date and time, eight-character sample identification number, and fish species and length. All entries are made in indelible ink, and they coincide with field sampling and whole fish sample information on the Sample Collection Form.

Proper chain-of-custody procedures are necessary for tracking sample possession from either Great Lake or mid-Atlantic river sampling locations to the fish sample preparation laboratory in Owings Mills, MD and from the sample preparation laboratory to the laboratory for mercury analysis in Kelso, WA or the laboratory for selenium and percent solid analyses in Bothell, WA. A Chain-of-Custody Form (Appendix A, Exhibit 9) accompanies each cooler containing whole fish or fillet plug samples during transport or shipment of the samples to the Tetra Tech laboratory in Owings Mills, MD. A Chain-of-Custody Label (Appendix A, Exhibit 10) seals each sample cooler after packing operations are completed in the field, and it includes the signature of the sampler and the date and time sealed. All entries on the Chain-of-Custody Forms are made in ink, and Chain-of-Custody Label entries are made in indelible ink. The Field Team Leader must notify the laboratory of any incoming delivery of samples (i.e., coolers). If the coolers are being shipped via an overnight air delivery service, the Field Team Leader must

provide the airbill tracking number to the sample preparation laboratory and to the EPA Project Manager.

Sampling activities will conclude with the development of the Fish Sample Master Spreadsheet (a detailed list of samples and sample information) by Tetra Tech and review of the master spreadsheet by the EPA Project Manager. The Fish Sample Master Spreadsheet includes waterbody, site identification, location (latitude and longitude), date, species, length (mm, total length), fish number, sample type, and sample number information for each sample. Tetra Tech maintains a file as a repository for information used in the preparation of the Fish Sample Master Spreadsheet and other study deliverables throughout the duration of the study. It includes the following information:

- any documents prepared for the study,
- contract and work assignment information,
- project QAPP,
- results of technical reviews, data quality assessments, field observations, and audits,
- communication records (memoranda; letters; meeting minutes; and all written correspondence between Tetra Tech, EPA, and other project team personnel, charter captains, or others),
- maps, photographs, and drawings, and
- studies, reports, and documents pertaining to the project.

The major deliverable requirements associated with the fish sample preparation laboratory include the following:

- The fish sample preparation laboratory must prepare and submit a weekly progress report to the EPA Fish Plug Evaluation Study Project Manager (based on fish preparation information recorded on the FPES Fish Sample Preparation Laboratory Bench Sheet in Appendix C for the mercury phase or Appendix E for the selenium phase) to document the status of fish sample preparation activities and provide information specified in the procedures described in Appendix B for the mercury phase and Appendix D for the selenium phase.
- The fish sample preparation laboratory must report the results of the rinsate analyses for mercury and the triplicate lipid results associated with each fish sample preparation batch (2 fish per batch) to the EPA Project Manager, along with the full set of lipid analysis results from the 60 fish samples (single lipid analysis results for 30 mercury phase fish and the average of the triplicate lipid results for the other 30 fish) collected for the mercury phase of the plug study.

- The sample preparation laboratory must report the results of the rinsate analyses for selenium and triplicate lipid results associated with each fish sample preparation batch (5 fish per batch) to the EPA Project Manager, along with the full set of lipid analysis results (single lipid analysis results for 24 selenium phase fish and the average of the triplicate lipid results for the other 6 fish) collected for the selenium phase of the plug study.

- The fish sample preparation laboratory must provide shipping information (tracking numbers, airbills, shipping forms, etc.) to the EPA Project Manager for tissue or rinsate samples sent to the analytical laboratory.

If any changes to this QAPP are required during the study, a memo will be sent to each person on the distribution list describing the change(s), following approval by the EPA Project Manager. Any and all memos announcing changes must be attached to the QAPP.

All documents and records prepared for this project will be maintained by Tetra Tech for the duration of the project, and retained for a period of five years following completion of the project (unless otherwise directed by EPA).

B. DATA GENERATION AND ACQUISITION

B1. Sampling Process Design

The Fish Plug Evaluation Study is designed to assess the comparability of mercury concentrations in fish fillet plugs vs. homogenized whole fillet tissue samples and to test the feasibility and applicability of fish fillet plug sampling and analysis for conducting compliance monitoring associated with EPA's fish tissue-based selenium water quality criterion. Following are the key elements of the Fish Plug Evaluation Study design:

- Fish sampling is being conducted in two waterbody types, the Great Lakes and U.S. rivers in the mid-Atlantic region. Lake Erie, Lake Michigan, and Lake Ontario are being targeted for Great Lakes fish sample collection, and the Anacostia River, the Potomac River, and the St. Lawrence River are being targeted for river fish collection.

- Individual whole fish samples are being collected from each waterbody type to provide plug and homogenized fillet tissue samples for mercury and selenium analyses.

- To provide tissue samples for mercury analysis, 10 specimens of three species each were collected from the designated Great Lakes and from the designated rivers. Target species for the Great Lakes are lake trout, walleye, and Chinook salmon. Target species for the rivers are largemouth bass, smallmouth bass, and blue catfish. This fish sampling effort, which was completed in September 2017, yielded 60 individual whole fish samples to be prepared for mercury analysis.

- Five replicates each of three types of fish tissue samples are prepared from each fish for mercury analysis (Figure 2): field-extracted fillet plug samples, lab-extracted fillet plug

samples, and lab-prepared homogenized whole fillet tissue samples, yielding 900 fish fillet tissue samples for mercury analysis (60 fish x 3 fillet sample types per fish x 5 replicates per fillet sample type = 900 fish fillet tissue samples).

- To provide tissue samples for selenium analysis, 5 specimens of three species each are being collected from the designated Great Lakes and from the designated rivers. Target species for the Great Lakes and rivers are the same as for mercury analysis (i.e., lake trout, walleye, and Chinook salmon for the Great Lakes and largemouth bass, smallmouth bass, and blue catfish for the rivers). This fish sampling effort will yield 30 individual whole fish samples to be prepared for selenium analysis.

- Four replicates each of three types of fish fillet samples are prepared from each fish for selenium analysis (Figure 3): field-extracted fillet plug samples, lab-extracted fillet plug samples, and lab-prepared homogenized whole fillet tissue samples, yielding 360 fish fillet tissue samples for selenium analysis (30 fish x 3 fillet sample types per fish x 4 replicates per fillet sample type = 360 fish fillet tissue samples).

- Field crew leaders hand deliver whole fish and field-extracted fillet plug samples to the Tetra Tech laboratory in Owings Mills, MD for interim storage and fish sample preparation. The 60 whole fish samples and 300 field-extracted plug samples collected for the mercury phase of the study during August and September 2017 were stored in freezers at the Tetra Tech laboratory prior to initiation of fish tissue sample preparation. The 15 Great Lakes whole fish samples and 60 pairs of field-extracted fillet plug samples (a double-plug sample for selenium analysis and a single-plug sample for percent solids analysis) were collected for the selenium phase of the study in June 2018. The remaining 15 selenium phase whole fish samples and 60 pairs of field-extracted fillet plug samples from rivers are scheduled for collection in July 2018. All 30 selenium phase whole fish samples and 120 pairs of field plug samples are stored in freezers at the Tetra Tech laboratory prior to initiation of fish tissue sample preparation and/or sample analysis

- The designated type and number of fillet tissue samples are analyzed for mercury and selenium as noted above for the two phases of the study. In addition, homogenized whole fillet tissue samples from the 90 fish collected for the study are analyzed for lipids (single lipid analysis for 30 mercury phase fish and 24 selenium phase fish and triplicate lipid analysis for 30 mercury phase fish and 6 selenium phase fish, which are reported as average lipid values). Details for lipid analysis of these fillet samples are provided in this QAPP Revision 2. All of the fillet tissue samples (field-extracted plugs, lab-extracted plugs, and homogenized whole fillet tissue samples) from each of the 30 fish collected for the selenium phase of the study are also analyzed for percent solids in order to provide selenium results on the dry-weight basis specified for the selenium water quality criterion. To provide tissue mass for the solids measurements, an additional 4 field-extracted plugs and 4 lab-extracted plugs are collected as single-plug samples. Procedures and requirements for analyzing Fish Plug Evaluation Study fillet tissue samples for mercury (total), selenium (total) and percent solids are provided in a separate sample analysis QAPP (USEPA 2018).

Figure 2. Fish Plug Evaluation Study (FPES) Field Sample Collection Summary for the Mercury Phase

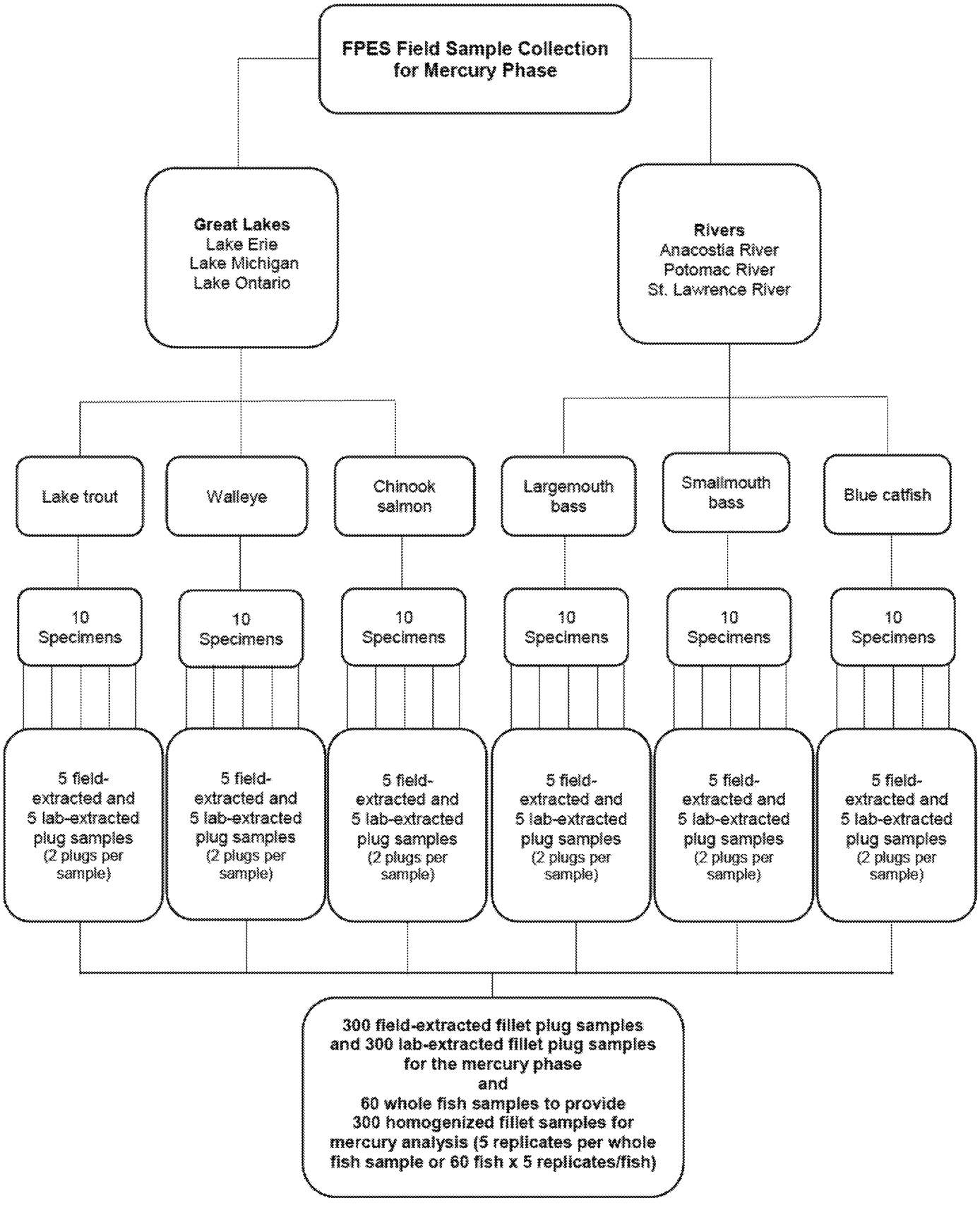
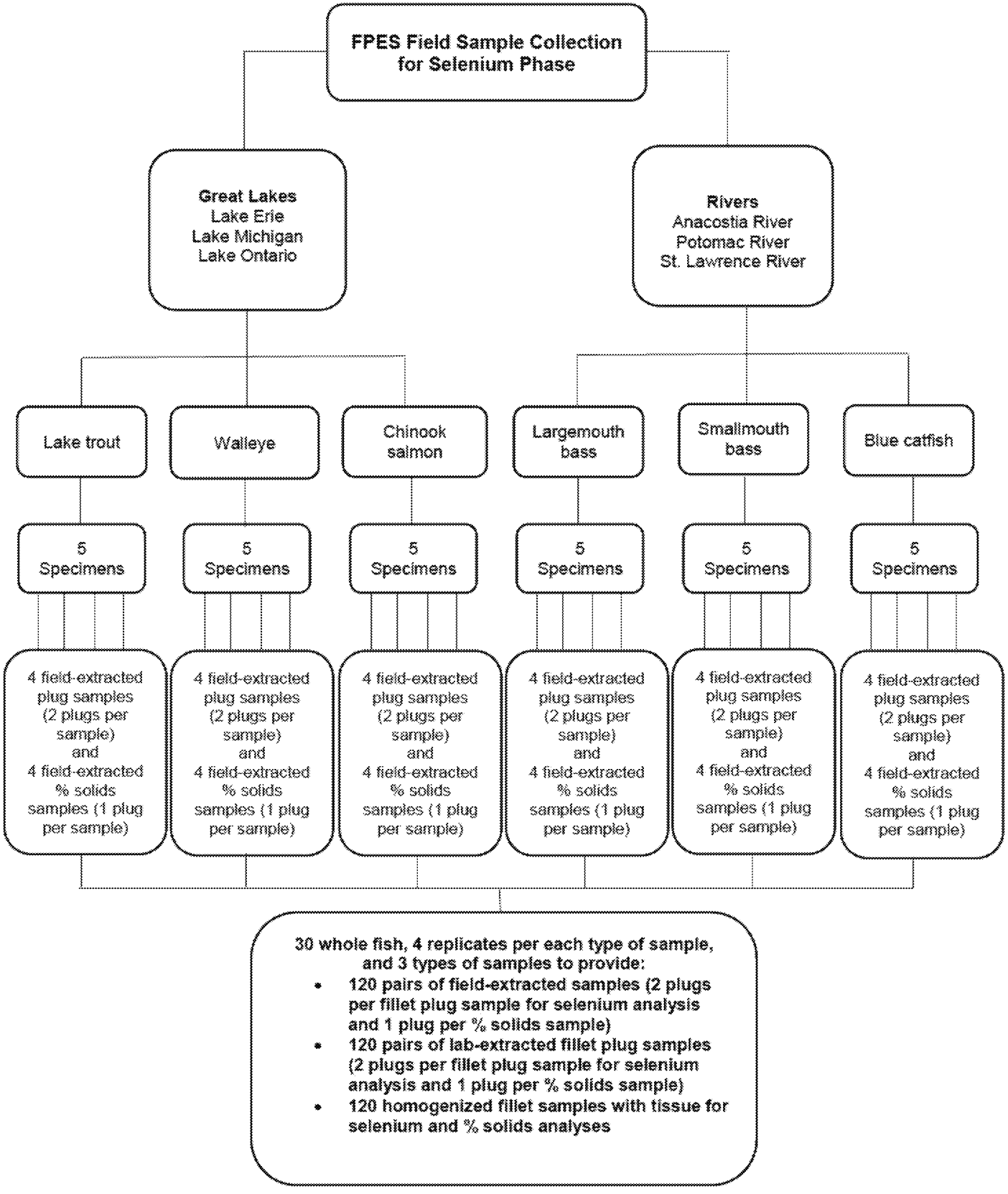


Figure 3. Fish Plug Evaluation Study (FPES) Field Sample Collection Summary for the Selenium Phase



B2. Sampling Methods

The Fish Plug Evaluation Study field objective is for sampling teams to obtain:

- 60 whole fish and 300 field-extracted fillet plug samples for the mercury phase of the study, and
- 30 whole fish and 120 pairs of field-extracted double-plug and single-plug fillet samples for the selenium phase of the study.

To provide tissue samples for mercury analysis, 10 specimens of three species each were collected from the designated Great Lakes and designated rivers (Figure 2). Target species for the Great Lakes are lake trout, walleye, and Chinook salmon. Target species for the rivers are largemouth bass, smallmouth bass, and blue catfish. This fish sampling effort, which was completed in September 2017, yielded 60 individual whole fish samples to be prepared for mercury analysis. For selenium analysis, 5 specimens of each of the six target species mentioned above were collected from the three designated Great Lakes in June 2018 and are scheduled for collection from the three designated rivers in July 2018, yielding 30 individual whole fish samples (Figure 3). Fish are collected by the field sampling teams, field-extracted fillet plugs are removed from the fish onsite, and those whole fish samples and plugs are transported to the Tetra Tech laboratory in Owings Mills, MD (where lab plugs and fillets are removed, and fillets are homogenized). The equipment selected for fish collection is at the discretion of the field sampling team and subject to weather and water conditions at the time of sampling, but it is anticipated that electrofishing will be used for river sampling and angling (hook and line) used in the Great Lakes. Field sampling teams must be qualified, experienced, and trained on the safe and effective use of each gear type selected. Field sampling procedures and requirements for collecting, handling, and shipping Fish Plug Evaluation Study whole fish samples and field-extracted fillet plug samples are specified in Appendix A.

B2.1 Whole Fish Sampling

Field sampling teams must assemble an array of active gear types (e.g., electrofishers and hook-and-line equipment) to ensure the collection of the desired target numbers and species of whole fish samples for both the mercury and selenium phases of the study (Figures 2 and 3, respectively). As soon as fish are obtained via active collection methods they should be identified to species. Species identification must be conducted only by experienced personnel with knowledge of the taxonomy of species in the target waterbodies. Non-target species collected by the field sampling team are immediately returned to the source water. As fish are collected in the field and retained as whole fish samples for the Fish Plug Evaluation Study, a GPS unit is used to identify the specific sampling coordinates (i.e., latitude and longitude) for each particular target fish specimen. The selected target fish specimens are rinsed in ambient water to remove any foreign material from the external surface, handled using clean nitrile gloves, and placed in clean holding containers (livewell, buckets, etc.) to prevent contamination. Each fish of the selected target species is measured to determine total body length (mm), which is the length from the anterior-most part of the fish to the tip of the longest caudal fin ray (when the lobes of the caudal fin are depressed dorsoventrally). The species (common name), specimen

length, sampling location, collection method, and a unique eight-character sample ID number must be recorded on the Fish Plug Evaluation Study Sample Collection Form (Appendix A).

The field objective is for sampling teams to obtain ten individuals of a target species from each sampling site (i.e., from each target river or Great Lake) for the mercury phase of the study and five individuals of a target species from each sampling site for the selenium phase of the study. The fish should be of a suitable size to provide adequate biomass for all plug and fillet samples needed for the Fish Plug Evaluation Study (i.e., obtain a minimum of 140 g of fillet tissue for both types of samples, which is equivalent to 5 oz). The selection process for whole fish samples are based on the following criteria:

- 10 adult individuals of the target species are available from the sampling site for mercury analysis during the initial mercury phase of the study and 5 individuals of the target species are available from the sampling site for selenium analysis during the subsequent selenium phase of the study,

- all specimens satisfy legal requirements of harvestable size for the sampled river or Great Lake, or at least are of a size large enough to provide all plug and fillet samples needed for the Fish Plug Evaluation Study if no legal harvest requirements are in effect.

Clean nitrile gloves must be worn during the entire sample handling process, beginning with removing the fish from the clean holding containers (livewell, buckets, etc.). After initial processing to determine species and size, field-extracted fillet plug samples are removed from each fish as described in Section B2.2, and gloves must be changed before handling each subsequent fish specimen for plug extraction. Following that plug extraction process, the whole fish samples are individually wrapped in heavy-duty aluminum foil (i.e., solvent-rinsed, oven-baked sheets). For specimens with sharp fins, spines may be broken (via gloved hands or with the use of a tool covered with the solvent-rinsed aluminum foil) to prevent perforation of the wrapping materials. A Whole Fish Label (Appendix A) is prepared for each aluminum foil-wrapped specimen. Each foil-wrapped fish is placed into waterproof plastic tubing that is cut to size to fit the specimen (i.e., heavy-duty food grade polyethylene tubing), and each end of the tubing is sealed with a plastic cable tie. A completed Whole Fish Label is affixed to the outside of the food grade tubing with clear tape and secured by taping around the entire fish (so that tape sticks to tape). Once packaged, samples are immediately placed on dry ice for transport or shipment. If samples are carried back to an interim location to be frozen before shipment, wet ice can be used to transport wrapped and labeled whole fish samples in coolers to that location. The whole fish sample shipment process is detailed in Section B2.3 and Appendix A.

B2.2 Field-Extracted Fillet Plug Sampling

Field-extracted plug samples are removed from the whole fish described in Section B2.1. Field sampling teams are responsible for preparing Field Plug Labels (Appendix A) for the sample, and ensuring that the label information matches the information recorded on the Fish Plug Evaluation Study Sample Collection Form. Each label is affixed to a sterile 20-milliliter scintillation vial and covered with clear tape. Clean nitrile gloves must be worn during the entire field plug extraction process. The process begins with the removal of scales (using a sterile disposable scalpel) from a small area on the left side dorsal area of the fish between the dorsal fin

and the lateral line. An 8-mm biopsy punch is inserted into the dorsal muscle of the fish through the scale-free area with a slight twisting motion, cutting the skin and muscle tissue. Once full depth penetration of the punch is achieved, it is bent or tilted to break off the end of the fillet plug. The full depth of the punch must be filled with muscle tissue to ensure collection of sufficient plug biomass for mercury or selenium analysis. A pipette bulb is then placed on the handle end of the biopsy punch and squeezed to force the plug into a sterile 20-milliliter scintillation vial. This process is repeated so that each field-extracted plug sample for both the mercury phase and selenium phase contains two plugs for metal analyses and the percent solids plug samples for the selenium phase contain one plug, before closing the cap on the scintillation vial.

Each field-extracted fillet plug sample vial is placed into a small bubble wrap bag and a sealed plastic bag before being placed immediately on dry ice or in a freezer for storage before transport or shipment. The nitrile gloves, scalpel, and biopsy punch must be disposed after the preparation of each series of plug samples from an individual fish. For the mercury phase of the study, this field plug extraction and packaging process needs to be repeated on the same fish 4 additional times (for a total of 5 field-extracted fillet plug samples). Each sample (2 plugs per sample) must be placed in a separate 20-milliliter scintillation vial. For the selenium phase, the field plug extraction process is repeated on the same fish 3 additional times (for a total of 4 field-extracted fillet plug samples), and again, each sample (2 plugs per sample) is placed in a separate 20-milliliter scintillation vial. An additional field-extracted plug sample will be collected from each fish during the selenium phase of the study for percent solids analysis (consisting of only 1 plug per sample). Frozen field-extracted plug samples are shipped on dry ice or hand delivered to Tetra Tech in Owings Mills, MD according to the process described in Section B2.3.

B2.3 Sample Shipment

In preparing whole fish and field-extracted fillet plug samples for shipping, field teams record sample number, species name, specimen length, sampling location, and sampling date and time on a Chain-of-Custody Record form. Each scintillation vial containing field-extracted fillet plug samples is placed into a small bubble wrap bag and sealed in a plastic bag, and is immediately frozen or placed on dry ice for storage before transport or shipment. Frozen field-extracted plug samples are packed in coolers on dry ice (i.e., a minimum of 30 pounds) and shipped via priority overnight air delivery service or hand delivered to Tetra Tech in Owings Mills, MD within one week of collection. Fish plug samples may be batch shipped (a maximum of 20 plug samples per cooler), but samples must be kept frozen at $\leq 20^{\circ}C$ until shipped on dry ice or hand delivered.

Each whole fish sample is wrapped in a sheet (or sheets) of solvent-rinsed, oven-baked aluminum foil, with the dull side in. Individual foil-wrapped specimens are placed into a length of food-grade polyethylene tubing, each end of the tubing is sealed with a plastic cable tie, and a fish specimen label is affixed to the outside of the food-grade tubing with clear tape. Each sample is then immediately frozen or placed on dry ice for storage before transport or shipment. Frozen whole fish samples are packed in coolers on dry ice (i.e., a minimum of 50 pounds) and shipped via priority overnight air delivery service or hand delivered to Tetra Tech in Owings Mills, MD within one week of collection.

A Chain-of-Custody Record form is completed for each field plug and whole fish sample cooler. All entries must be in black ink and coincide with specimen/sample information on the Fish Plug Evaluation Study Sample Collection Forms and the corresponding Whole Fish Labels or Field Plug Labels. One copy of the Chain-of-Custody Form is retained by the field sampling team, and all other copies are placed in a waterproof bag and enclosed in the shipping cooler.

Field crews are directed to pack all whole fish and field plug samples on dry ice in sufficient quantities to keep samples frozen for up to 48 hours (50 pounds are required for whole fish coolers and 30 pounds are required for field plug coolers), to seal each sample cooler with packaging tape and a Custody Seal (Appendix A), and to ship them via priority overnight air delivery service (e.g., FedEx), so that they arrive at Tetra Tech in less than 24 hours from the time of sample collection. Alternatively, field crews may transport whole fish samples on wet or dry ice (depending on the distance) to an interim location where the fish samples are frozen and stored before overnight shipping or hand delivery to the Tetra Tech Laboratory in Owings Mills, MD on dry ice as described above.

Tetra Tech at Owings Mills, MD is serving as EPA's interim storage facility for the Fish Plug Evaluation Study field-extracted fillet plug samples and whole fish samples and as the laboratory for preparation of lab-extracted plug and homogenized fillet tissue samples. Tetra Tech staff are responsible for receiving and examining the fish samples as they arrive and before they are stored in a walk-in freezer at the laboratory. The specific procedures that the laboratory staff follow upon receipt of all samples are described in Section B3. Tetra Tech staff are also responsible for forwarding electronic copies of the sample records to the EPA Project Manager.

B3. Sample Receipt and Inspection

This section describes the procedures for receiving and inspecting Fish Plug Evaluation Study whole fish samples and field-extracted fillet plug samples when coolers containing these samples are delivered to the Tetra Tech laboratory in Owings Mills, MD, which has been designated for fish sample preparation. At the discretion of the Field Team Leader, the whole fish samples and field fillet plug samples may be either transported by field sampling personnel or shipped via priority overnight air service to the Owings Mills, MD laboratory. Sample coolers must be opened and inspected at the laboratory promptly on receipt. The sample custodian at the Tetra Tech laboratory will:

- Check that each cooler has arrived undamaged and verify that samples are still frozen and in good condition.

- Check the temperature of one of the samples in the cooler using either a thermometer that reads to at least -20 degrees Celsius (°C) or an infra-red (IR) temperature "gun," and record the reading on the Chain-of-Custody Form.

- Verify that all associated paperwork is complete, legible, and accurate.

- Compare the information on the label on each individual fish specimen and each fillet plug sample vial to the Chain-of-Custody Form for each composite and verify that each sample was included in the shipment and is properly wrapped and labeled.

- Notify the EPA Project Manager about receipt of samples on the day of delivery and report any discrepancies in the paperwork identified above.

- Provide the EPA Project Manager with a copy of the field form for each sample and the Chain-of-Custody Form (via email).

- Transfer the samples with their original labels to the freezer for interim storage.

- Retain copies of all the shipping airbills associated with sample coolers delivered to the laboratory by an overnight express air service.

The sample preparation laboratory notifies the EPA Project Manager immediately about any problems encountered upon receipt of samples. Problems involving sample integrity (i.e., sample condition) and conformity for either the whole fish samples or the field fillet plug samples must be reported to the EPA Project Manager in writing (i.e., by email) within 24 hours following sample receipt and inspection.

After completing sample receipt and inspection, the sample preparation laboratory must store the whole fish and fillet plug samples in an onsite freezer at temperatures less than or equal to -20 °C until they are ready for sample preparation (whole fish samples) or shipment to the analytical laboratory designated for either mercury or selenium analysis (field-extracted and lab-extracted fillet plug samples for mercury and selenium analyses, percent solids samples for analysis only during the selenium phase, and homogenized fillet tissue samples for both mercury and selenium analyses).

B4. Fish Sample Preparation and Analytical Methods

This section describes Fish Plug Evaluation Study fish sample preparation methods (i.e., methods for extracting plug samples in the laboratory and for preparing homogenized fillet tissue samples). It also describes methods for analysis of lipids in ground fillet tissue samples for homogeneity testing and lipid content and for analysis of mercury and selenium in equipment rinsate samples to test the adequacy of equipment cleaning. Lipid analysis of ground fillet tissue samples for homogeneity testing and mercury and selenium analysis of equipment rinsate samples for testing sufficient equipment cleaning are conducted as part of the QC procedures for fish sample preparation. Analytical method requirements for mercury analysis of mercury phase fillet samples (i.e., field-extracted plug samples, lab-extracted plug samples, and homogenized fillet tissue samples) and for selenium analysis of selenium phase fillet samples (i.e., field-extracted plug samples, field-extracted percent solids samples, lab-extracted plug samples, and homogenized fillet tissue samples) are described in the Fish Plug Evaluation Study sample analysis QAPP (USEPA 2018).

B4.1 Fish Sample Preparation

The laboratory at Tetra Tech's Biological Research Facility in Owings Mills, MD is the fish sample preparation laboratory for the Fish Plug Evaluation Study. During the mercury phase of the study, trained laboratory staff are responsible for extracting 5 plug samples (2 plugs per

sample) from the individual fish samples (60 total) before beginning preparation of the homogenized fillet tissue samples. During the selenium phase, the sample preparation laboratory staff are responsible for extracting 4 fillet plug samples (2 plugs per sample) and 4 percent solids plugs (1 plug per sample) from the individual fish samples (30 total) in the laboratory before beginning preparation of the homogenized fillet tissue samples. For preparation of ground fillet tissue samples during both the mercury phase and the selenium phase, these laboratory personnel are responsible for filleting each individual fish sample, homogenizing the fillet tissue from each whole fish sample, preparing the required number of fish tissue aliquots for analysis and archive, shipping the fish tissue aliquots for each analysis to the designated analytical laboratory, and storing archived fish tissue samples in a freezer at their facility. The specific procedures for lab-extracted plug and fillet tissue sample preparation activities for the mercury phase are summarized below and fully described in Appendix B. Procedures for laboratory fillet tissue sample preparation for the selenium phase are summarized below and described in detail in Appendix D.

Fillet Plug Extraction for Mercury Phase

Before beginning fillet plug extraction, trained laboratory technicians weigh each whole fish sample to the nearest gram, rinse the single fish with deionized water, place it on a clean glass cutting board, and remove all scales. Using a biopsy punch, the lab technicians extract 5 fillet plug samples (2 plugs per sample) from each fish (i.e., 10 plugs are extracted to form 5 plug samples) and place each of the 5 fillet plug samples in glass scintillation vials (one plug sample per vial). The vials are weighed and labeled before being transferred to a freezer for storage at less than or equal to -20°C.

Fillet Plug Extraction for Selenium Phase

Before beginning fillet plug extraction, trained laboratory technicians weigh each whole fish sample to the nearest gram, rinse the single fish with deionized water, place it on a clean glass cutting board, and remove all scales. Using a biopsy punch, the lab technicians extract 4 double-plug fillet samples from each fish for selenium analysis and 4 single-plug fillet samples from each fish for percent solids analysis. The technicians place each of the 8 fillet plug samples in glass scintillation vials. The vials are weighed and labeled before being transferred to a freezer for storage at less than or equal to -20°C.

Homogenized Fillet Sample Preparation for Mercury and Selenium Phases

The filleting process involves removing the fillet (with skin on and "belly flap" or ventral muscle attached) from both sides of each individual whole fish sample. The two fillets from each individual fish sample are weighed to the nearest gram (wet weight) before they are homogenized together.

An electric meat grinder is used to prepare each homogenized fillet sample. The entire fillets (with skin and belly flap) from both sides of each whole fish sample are homogenized, and the entire homogenized volume is used to prepare the fillet tissue. Grinding of the fillet tissue is repeated until the tissue consists of a uniform color and finely ground texture. Homogeneity is confirmed by conducting triplicate analyses of the lipid content in one of every two fish samples

represented in each mercury phase fish sample preparation batch (i.e., triplicate lipid analyses of homogenized fillet tissue samples are conducted for 30 of the 60 total individual fish samples collected for the mercury phase of the study) and in one of every 5 fish represented in each selenium phase fish sample preparation batch (i.e., triplicate lipid analyses of homogenized fillet tissue samples are conducted for 6 of the 30 total individual fish samples collected during the selenium phase of the study). The collective weight of the homogenized fillet tissue from each fish sample is recorded to the nearest gram (wet weight) after processing. For the mercury phase, the sample preparation laboratory prepares 5 fillet tissue sample aliquots containing a mass of 5 - 10 grams (i.e., 5 replicates of the homogenized fillet tissue from each fish) for mercury analysis according to the specifications in the fish sample preparation procedures in Appendix B. For the selenium phase, the sample preparation laboratory prepares 4 fillet tissue sample aliquots containing a mass of 20 - 25 grams (i.e., 4 replicates of the homogenized fillet tissue from each fish) for selenium and percent solids analyses according to the specifications in the fish sample preparation procedures in Appendix D.

Mercury Phase Fish Sample Preparation Batches

Each fish sample preparation batch consists of two individual fish samples for a total of 30 fish sample preparation batches to be processed from the 60 individual fish samples collected during the mercury phase of the Fish Plug Evaluation Study. The fish sample preparation batches are formed by assigning two fish to each batch based on the chronological order in which the fish samples were collected, beginning with the oldest samples first. The fish processing instructions are the same for every fish sample preparation batch. Processing of each fish sample preparation batch involves the removal of 5 replicate plug samples (2 plugs per plug sample) and the preparation of 5 replicate homogenized fillet tissue samples from each of the two fish in the batch, which produces a total of 10 lab-extracted plug samples and 10 homogenized fillet tissue samples per batch.

Selenium Phase Fish Sample Preparation Batches

Each fish sample preparation batch consists of five individual fish samples for a total of 6 fish sample preparation batches to be processed from the 30 individual fish samples collected during the selenium phase of the Fish Plug Evaluation Study. The fish sample preparation batches are formed by assigning five fish to each batch based on the chronological order in which the fish samples were collected, beginning with the oldest samples first. The fish processing instructions are the same for every fish sample preparation batch. Processing of each fish sample preparation batch involves the removal of 4 replicate double-plug fillet samples (for selenium analysis) and 4 replicate single-plug fillet samples (for percent solids analysis) prior to the preparation of 4 replicate homogenized fillet tissue samples from each of the five fish in the batch. Each selenium fish sample preparation batch produces 20 paired lab plug samples and 20 homogenized fillet tissue samples for selenium and percent solids analyses.

Mercury Analysis Batches

A mercury analysis batch consists of the 20 fillet tissue samples generated during processing of a fish sample preparation batch. These 20 fillet tissue samples include the 10 lab-extracted plug samples (5 replicate samples per fish) and the corresponding 10 homogenized fillet tissue

samples (5 replicate samples per fish) from the 2 individual fish samples in a fish sample preparation batch. The fish sample preparation laboratory will produce 30 batches of fillet tissue samples (or 600 individual fillet tissue samples) to analyze for mercury. ALS Environmental in Kelso, WA is analyzing these fillet tissue samples for mercury. Analytical method requirements for mercury analysis of these samples are described in the Fish Plug Evaluation Study sample analysis QAPP (USEPA 2018).

Selenium Analysis Batches

There are two selenium analysis batches (20 samples per batch) produced from each selenium phase fish sample preparation batch. One consists of the paired double-plug and single-plug fillet samples for selenium analysis and percent solids analysis, respectively. The other selenium analysis batch consists of the 20 homogenized fillet tissue samples generated during processing of the fish sample preparation batch (4 replicate samples per fish from the 5 individual fish samples in a fish sample preparation batch). The fish sample preparation laboratory will produce 6 batches of paired lab fillet plug samples (or 120 paired lab plug samples) and 6 batches of fillet tissue samples (or 120 individual fillet tissue samples) to analyze for selenium and percent solids. Brooks Applied Labs in Bothell, WA is analyzing all of these fillet samples for selenium and percent solids. Analytical method requirements for selenium analysis and percent solids analysis of these samples are described in the Fish Plug Evaluation Study sample analysis QAPP (USEPA 2018).

B4.2 Lipid Analysis

The fish sample preparation laboratory procured the services of ALS Environmental in Kelso, WA to conduct one set of triplicate lipid analyses per fish sample preparation batch (see definition in Section B4.1) as described in Steps 35 and 36 of Appendix B for the mercury phase and Steps 36 and 37 of Appendix D for the selenium phase. As noted in Section B4.1, triplicate lipid analyses of ground fillet tissue samples for homogeneity testing are conducted for 30 of the 60 total individual fish samples collected for the mercury phase and for 6 of the 30 total fish samples collected for the selenium phase of this study. For the remaining 30 individual fish samples from the mercury phase and 24 fish samples from the selenium phase, a single homogenized fillet tissue sample is analyzed for lipid content.

Lipids are extracted from each fillet tissue sample using the EPA 3541/NOAA Method. This method is based on the procedure described in the Puget Sound Protocols (Bligh and Dyer 1959) and EPA's Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1 (USEPA 2000). This procedure is used to determine the lipid content in biological tissue. A homogenized fillet tissue sample aliquot (10 to 15 grams, wet weight) is extracted with organic solvent, the extract is evaporated using moderate heat, and the lipid weight is determined. Percent lipid content is calculated by dividing the lipid weight by the initial fillet tissue aliquot weight and multiplying that result by 100. The batch-specific lipid results for homogeneity evaluations are reviewed initially by Tetra Tech and independently by CSRA against the QC specifications detailed in Section B5.2.

B4.3 Mercury Rinsate Analysis

The fish sample preparation laboratory prepares one set of rinsate samples per fish sample preparation batch (see definition in Section B4.1) for mercury analysis as described in Steps 32 through 34 of Appendix B. This set of rinsate samples consists of one deionized (DI) water equipment rinsate sample and one DI water blank sample. The requirement to prepare a set of rinsate samples for each fish sample preparation batch will generate 30 sets of rinsate samples (or 60 individual aqueous samples) for mercury analysis.

The fish sample preparation laboratory procured the services of ALS Environmental to analyze the 30 sets of rinsate samples for mercury. This laboratory is conducting mercury analysis of the rinsate samples using EPA Method 245.1 (USEPA 1994a). This method was developed to measure total mercury (organic and inorganic) in drinking, surface, saline, and ground waters, as well as in domestic and industrial wastes. The flameless atomic absorption procedure is a physical method based on the absorption of radiation at 253.7 nanometers (nm) by mercury vapor. Mercury is first reduced to its elemental state using a potassium permanganate digestion procedure. The samples/standards and a stannous chloride reagent are then pumped into the mercury analyzer and mixed. Argon gas is introduced into the solution stream. The gas and liquid stream is transferred to the gas/liquid separator where the gas and liquid phases are separated. The liquid waste is drained off and the gas (containing mercury) is pumped to the absorption cell. The absorption cell is positioned in the light path of the mercury lamp. Absorbance (peak height) is measured as a function of mercury concentration and recorded as ppb of mercury. Results of the batch-specific mercury rinsate analyses are reviewed initially by Tetra Tech and independently by CSRA against the QC specifications detailed in Section B5.2.

B4.4 Selenium Rinsate Analysis

The fish sample preparation laboratory prepares one set of rinsate samples per fish sample preparation batch (see definition in Section B4.1) for selenium analysis as described in Steps 33 through 35 of Appendix D. This set of rinsate samples consists of one deionized (DI) water equipment rinsate sample and one DI water blank sample. The requirement to prepare a set of rinsate samples for each fish sample preparation batch will generate 6 sets of rinsate samples (or 12 individual aqueous samples) for selenium analysis.

The fish sample preparation laboratory will procure the services of an analytical laboratory to analyze the 6 sets of rinsate samples for selenium. This laboratory will conduct selenium analysis of the rinsate samples using EPA Method 200.8 Revision 5.4 (USEPA 1994b). This method determines selenium in aqueous samples using inductively coupled plasma - mass spectrometry (ICP/MS) after performing an acid digestion procedure that is also described in the method. At least 20-mL of sample solution is pneumatically nebulized into a radio-frequency plasma where ionization occurs. The ions are extracted from the plasma through a differentially pumped vacuum interface and separated based on their mass-to-charge ratio by a quadruple mass spectrometer. Separated ions are detected by an electron multiplier or Faraday detector and the ion information processed by a data handling system. QC samples and acceptance criteria for selenium analysis of rinsates are detailed in Section B5.4.

B5. Quality Control Requirements

Data quality is addressed, in part, by consistent performance of the valid sample collection procedures documented in Appendix A. It is enhanced by the training and experience of project staff (Section A8) and documentation of project activities (Section A9). This QAPP was distributed to all field sampling personnel. Orientation and training sessions were conducted to distribute project materials and discuss field procedures. Field team leaders were required to read the QAPP, verify in writing (via email) that they read the QAPP, and participate in the fish sample collection training. After the training was completed, Tetra Tech prepared and submitted a summary of the training and a list of the training participants to the EPA Project Manager.

The project-specific QC procedures associated with the mercury phase fish sample preparation process in the laboratory include extraction of fillet plug samples, preparation of homogenized fillet tissue samples, and analytical testing for mercury in equipment rinsate samples, as well as triplicate lipid analyses of homogenized fillet tissue samples to test for homogeneity. The QC procedures are performed for one of every two fish samples represented in each fish sample preparation batch (see batch description in Section B4.1). Project-specific QC procedures for the selenium phase fish sample preparation process in the laboratory include extraction of fillet plug samples, preparation of homogenized fillet tissue samples, and analytical testing for selenium in equipment rinsate samples, as well as triplicate lipid analyses of homogenized fillet tissue samples to test for homogeneity. The QC procedures are performed for one of every five fish samples represented in each fish sample preparation batch (see batch description in Section B4.1).

B5.1 Fish Fillet Plug Samples

Quality control for extraction of fish fillet plug samples is addressed, in part, by laboratory staff adhering to the requirements in the fish fillet plug procedures, which are described in Appendix B and Appendix D. In addition, specialized training is provided for all laboratory technicians who are responsible for extracting fillet plug samples and preparing homogenized fillet tissue samples. Through training, these laboratory staff become proficient in the tasks required for fillet plug sample extraction and in the use of specified equipment for extracting and storing plug samples (Appendix B, Steps 10-16, for mercury phase procedures; Appendix D, Steps 10-17, for selenium phase procedures).

B5.2 Homogenized Fish Fillet Samples

Lipid content analysis is used as a surrogate to confirm homogeneity of the homogenized fish fillet samples that are prepared in the Tetra Tech Biological Research Facility laboratory. ALS Environmental in Kelso, WA is under contract to Tetra Tech to conduct triplicate lipid analyses of ground fillet tissue aliquots from one fish in each fish sample preparation batch and use the lipid content of those 3 fillet tissue aliquots to confirm that the ground fillet tissue is homogeneous. The total mass required for triplicate lipid testing of each of the 30 fish and 6 fish from the corresponding number of fish sample preparation batches in the mercury phase and selenium phase, respectively, is 30 to 35 g (an additional 20 to 25 g of fillet tissue mass than is required for lipid analysis of a single fillet tissue aliquot from each remaining fish sample in a fish sample preparation batch (one fish for mercury and four fish for selenium)). Therefore, for

the 30 fish samples from the mercury phase of the study and the 6 fish samples from the selenium phase that are selected for homogeneity testing, the fish sample preparation laboratory prepares this single larger aliquot (30 to 35 g) of homogenized fillet tissue mass for lipid analysis following the specific procedures described in Appendix B and Appendix D for placing the aliquots in containers, labeling them, and storing them in a freezer (refer to Step 27 in Appendix B and Step 28 in Appendix D). All homogenized fillet tissue aliquots for lipid analysis are shipped on dry ice and under chain-of-custody to ALS Environmental using the procedures described in the EPA 3541/NOAA Method. The results of the homogeneity testing are delivered to Tetra Tech for their initial review and forwarded to the EPA Project Manager for independent review by CSRA and EPA.

From the triplicate lipid results, Tetra Tech calculates the mean lipid content (in percent), the standard deviation (SD), and the relative standard deviation (RSD) using the formulae below, or the corresponding functions in Excel.

$$\text{mean } \%\text{lipids} = \frac{\sum_{i=1}^{3} (\%\text{lipids})_i}{3}$$

$$SD = \sqrt{\frac{\sum_{i=1}^{3} (\%\text{lipids}_i - \text{mean lipids})^2}{2}}$$

$$RSD = \frac{SD}{\text{mean}}$$

If the RSD of the triplicate lipid results is less than or equal to 15%, then EPA, CSRA, and Tetra Tech judge the homogenization effort to be sufficient for all the replicate homogenized fillet tissue samples (10) in each mercury analysis batch (refer to Step 36 in Appendix B) and in each selenium analysis batch (refer to Step 37 in Appendix D).

During the mercury phase, the fish sample preparation laboratory may continue to process up to 5 additional fish sample preparation batches (that each produce 20 fillet samples for a mercury analysis batch, which includes 10 lab-extracted fillet plug samples and 10 homogenized fillet tissue samples). However, the sample preparation laboratory may not continue to process batches beyond those 6 fish sample preparation batches until receiving notification from the EPA Project Manager that review of homogeneity test results from the initial batch in the set of 6 fish sample preparation batches is complete and the results are deemed satisfactory.

During the selenium phase, the fish sample preparation laboratory may continue to process up to 3 additional fish sample preparation batches. However, the sample preparation laboratory may not continue to process batches beyond those 4 fish sample preparation batches until receiving notification from the EPA Project Manager that review of homogeneity test results from the initial batch in the set of 4 fish sample preparation batches is complete and the results are deemed satisfactory.

B5.3 Mercury Analysis of Rinsate Samples

The Tetra Tech fish sample preparation laboratory prepares one set of DI water equipment rinsate and blank samples during processing of each of the 30 fish sample preparation batches (Appendix B, Step 34). ALS Environmental is under contract to Tetra Tech to analyze each set of rinsate samples for total mercury using EPA Method 245.1, which is a cold-vapor atomic absorption procedure applicable to water samples (Section B4.3). The 30 sets of rinsate samples are analyzed individually (not in batches of up to 20 samples) to provide timely feedback on the cleanliness of the homogenization equipment.

EPA Method 245.1 requires daily instrument calibration and analysis of two quality control samples, an instrument blank and a laboratory control sample. The rinsates are prepared in reagent water, so there is little chance of a "matrix effect." Each laboratory control sample, which is also prepared in reagent water, provides sufficient information on the performance of the method and the laboratory. Both of the quality control samples associated with a set of rinsate samples are analyzed for mercury with the set of rinsate samples. The QC sample requirements, including the acceptance criteria and corrective actions, are summarized in Table 2 below.

Table 2. QC Samples and Acceptance Criteria for Mercury Analysis of Rinsates

Quality Control Sample	Frequency	Acceptance Criteria
Instrument blank	With each rinsate sample	Result must be less than the MDL. Otherwise, redigest and reanalyze the rinsate sample.
Laboratory control sample	With each rinsate sample	80 - 120% recovery of mercury. Otherwise, correct instrumental problems, and redigest and reanalyze the rinsate sample.

The batch-specific rinsate results are reviewed initially by Tetra Tech and forwarded to the EPA Project Manager for independent review by CSRA. The rinsate results are evaluated based on the mass of mercury detected and the assumption that all of the apparent contamination could be transferred to a nominal 50-g mass of homogenized tissue. If the results for the rinsate samples are below the acceptance limit for mercury (i.e., 0.2 µg/L for total mercury) based on the method detection limit for an aqueous sample, then Tetra Tech and CSRA will judge the equipment cleaning effort to be sufficient for all samples in that fish sample preparation batch and report the outcome of the review to the EPA Project Manager.

Rinsate results for mercury above the reporting limit mentioned above may cause a need for corrective actions by the fish sample preparation laboratory. These corrective actions may include revisions to the sample preparation laboratory's equipment cleaning procedures, followed by a successful demonstration of the revised cleaning procedures through preparation and analysis of additional rinsate samples.

The fish sample preparation laboratory may continue to process up to five additional fish sample preparation batches (that each produce 20 fillet samples for a mercury analysis batch, which includes 10 lab-extracted fillet plug samples and 10 homogenized fillet tissue samples). However, the sample preparation laboratory may not continue to process batches beyond those six fish sample preparation batches until receiving notification from the EPA Project Manager

that review of rinsate test results from the initial batch in the set of six fish sample preparation batches is complete and the results are deemed satisfactory.

B5.4 Selenium Analysis of Rinsate Samples

The Tetra Tech fish sample preparation laboratory prepares one set of DI water equipment rinsate and blank samples during processing of each of the 6 fish sample preparation batches (Appendix D, Step 35). A laboratory under contract to Tetra Tech analyzes each set of rinsate samples for total selenium using EPA Method 200.8 Revision 5.4 (section B4.4). The 6 sets of rinsate samples are analyzed individually (not in batches of up to 20 samples) to provide timely feedback on the cleanliness of the homogenization equipment.

EPA Method 200.8 requires daily instrument calibration and analysis of two quality control samples, an instrument blank and a laboratory control sample. The rinsates are prepared in reagent water, so there is little chance of a "matrix effect." Each laboratory control sample, which is also prepared in reagent water, provides sufficient information on the performance of the method and the laboratory. Both of the quality control samples associated with a set of rinsate samples are analyzed for selenium with the set of rinsate samples. The QC sample requirements, including the acceptance criteria and corrective actions, are summarized in Table 3 below.

Table 3. QC Samples and Acceptance Criteria for Selenium Analysis of Rinsates

Quality Control Sample	Frequency	Acceptance Criteria
Instrument blank	With each rinsate sample	Result must be less than the MDL. Otherwise, redigest and reanalyze the rinsate sample.
Laboratory control sample	With each rinsate sample	80 - 120% recovery of selenium. Otherwise, correct instrumental problems, and redigest and reanalyze the rinsate sample.

The batch-specific rinsate results are reviewed initially by Tetra Tech and forwarded to the EPA Project Manager for independent review by CSRA. The rinsate results are evaluated based on the mass of selenium detected and the assumption that all of the apparent contamination could be transferred to a nominal 650-g mass of homogenized tissue. If the results for the rinsate samples are below the acceptance limit for selenium (i.e., 8 µg/L for selenium) based on the method detection limit for an aqueous sample, then Tetra Tech and CSRA will judge the equipment cleaning effort to be sufficient for all samples in that fish sample preparation batch and report the outcome of the review to the EPA Project Manager.

Rinsate results for selenium above the reporting limit mentioned above may cause a need for corrective actions by the fish sample preparation laboratory. These corrective actions may include revisions to the sample preparation laboratory's equipment cleaning procedures, followed by a successful demonstration of the revised cleaning procedures through preparation and analysis of additional rinsate samples.

The fish sample preparation laboratory may continue to process up to three additional fish sample preparation batches (that each produce 20 homogenized fillet samples for one selenium analysis batch and another 20 lab-extracted plug samples for a second selenium analysis batch). However, the sample preparation laboratory may not continue to process batches beyond those four fish sample preparation batches until receiving notification from the EPA Project Manager that review of rinsate test results from the initial batch in the set of four fish sample preparation batches is complete and the results are deemed satisfactory.

B5.5 Percent Solids Analysis (Selenium Phase)

Because EPA's water quality criterion for selenium is expressed in terms of the dry-weight concentration of selenium in fish tissue, four additional field-extracted fillet plugs and four additional lab-extracted fillet plugs will be extracted from each individual fish collected during the selenium phase. Each plug is placed in a separate vial, according to Step 15 in Appendix D, and shipped to Brooks Applied Labs for percent solids analysis. The sample containers used for the homogenized whole fillet tissue samples will contain enough additional tissue to allow for solids analysis of each of the homogenized fillet tissue samples as well.

Brooks Applied Labs will determine the solids content of the samples by drying each sample to constant weight at 103 -105 °C, using Standard Method 2540G (APHA 2005). Those percent solids results will be converted to percent moisture results (e.g., % moisture = 100% - % solids) by CSRA during their data reduction efforts.

B6. Instrument/Equipment Testing, Inspection, and Maintenance Requirements

All field equipment is inspected prior to sampling activities to ensure that proper use requirements are met (e.g., boats or electrofishers are operating correctly, nets are without defects, etc.). Inspection of field equipment occurs well in advance of the field operation to allow time for replacement or repair of defective equipment, and the field team is equipped with proper backup equipment to prevent lost time on site. One member of each field team gathers and inspects all equipment on the equipment and supply list (Appendix A) prior to each sampling event.

There are no analytical instruments used in the preparation of the fillet tissue samples. However, the balances used to weigh the whole fish and the tissue sample aliquots are inspected daily and the homogenization equipment (meat grinder) is inspected when it is reassembled after cleaning between samples.

All analytical instrumentation associated with the fish fillet homogeneity (lipid) testing, rinsate (mercury and selenium), and percent solids analyses is inspected and maintained as described in the respective analytical methods and laboratory SOPs.

B7. Instrument Calibration and Frequency

Instruments and instrument calibrations are not required for the field sample collection activities associated with the Fish Plug Evaluation Study. The balances used to weigh the whole fish and

the tissue sample aliquots during sample preparation activities in the laboratory are calibrated daily (i.e., calibrations are verified at the beginning of each day on which the balances are used).

All analytical instrumentation associated with the fish fillet homogeneity (lipid) testing and with the rinsate and percent solids analyses is calibrated as described in the respective analytical methods and laboratory SOPs.

B8. Inspection/Acceptance of Supplies and Consumables

Careful and thorough planning is necessary to ensure the efficient and effective completion of the field sample collection and fillet tissue sample preparation tasks. General checklists of field and laboratory equipment and supplies are provided in Appendix A, Appendix B (for mercury), and Appendix D (for selenium), respectively. Sampling gear and all fish sample packaging and shipping supplies are provided by Tetra Tech. It is the responsibility of each field team to gather and inspect the necessary sampling gear prior to the sampling event and to inspect the sample packaging and shipping supplies before leaving for a sampling trip. Defective packaging and shipping supplies are discarded, and if necessary, the field team obtains replacement supplies from the Tetra Tech laboratory. It is the responsibility of the fish sample preparation laboratory technicians to procure, compile, and inspect the necessary fillet sample preparation equipment and supplies prior to commencement of fillet tissue sample preparation activities, and to inspect packaging and shipping supplies before fillet tissue samples are shipped to ALS Environmental in Kelso, WA for mercury analysis or Brooks Applied Labs in Bothell, WA for selenium analysis.

B9. Non-direct Measurements

Non-direct measurements are not required for this project.

B10. Data Management

Data management practices employed in this study are based on standard data management practices used for EPA's National Lake Fish Tissue Study and other OST fish contamination studies (e.g. 2010 Great Lakes Human Health Fish Tissue Study). The data management (i.e., sample tracking, data tracking, data inspection, data quality assessment, database development) procedures have been regularly applied to other technical studies by Tetra Tech and CSRA. These procedures are being employed because they are effective, efficient, and have successfully withstood repeated internal and external audits, including internal review by EPA Quality Staff, public review and comment, judicial challenge, and an audit by the Government Accountability Office.

Fish Sample Collection Data

Samples are documented and tracked through the use of Fish Plug Evaluation Study Sample Collection Forms, Field Plug Labels, Whole Fish Labels, and Chain-of-Custody Forms (Appendix A). The diligence of the field sampling teams in completion of the proper records is essential. Field teams are responsible for reviewing all completed forms and labels. Any corrections should be noted, initialed, and dated by the reviewer. Chain-of-Custody Forms are

prepared and replicated in the field, via multiple page "carbonless copy" forms. The sampler retains one copy of the Chain-of-Custody Form, and the remaining copies are delivered to the sample preparation laboratory with the samples. If whole fish samples and field-extracted plug samples are hand delivered, shipment of samples to the sample preparation laboratory (Section B2) must be conducted by an overnight air delivery service that provides constant tracking of shipments (e.g. FedEx).

The sample preparation laboratory retains one copy of each Fish Plug Evaluation Study Sample Collection Form and Chain-of-Custody Form. Tetra Tech performs a data QC check on the Fish Plug Evaluation Study Sample Collection Form and forwards the original form to the EPA Project Manager, along with documentation reporting the field data QC review (consistent with field data QC documentation provided for previous EPA fish tissue studies). All form copies produced and retained by Tetra Tech are maintained in a project file during the active phase of this project, and for a period of 5 years following completion of the project (unless otherwise directed by EPA).

Upon completion of sampling activities, Tetra Tech develops a Fish Sample Master Spreadsheet based on information recorded by all field sampling teams on the Fish Plug Evaluation Study Sample Collection Forms. This field data is entered into an Excel spreadsheet to create the master spreadsheet. All data entries are checked for errors in transcription and computer input by qualified persons (minimum of two) who did not originally enter the data. If there is any indication that requirements for sample integrity or data quality have not been met, the Tetra Tech QA Officer is notified immediately (with an accompanying explanation of the problems encountered).

Fish Sample Preparation Data

The Tetra Tech sample preparation laboratory is required to maintain all records and documentation associated with the preparation of study samples (e.g., weekly reports containing tissue sample preparation data and tissue aliquot documentation), the analysis of lipids for homogeneity testing and lipid content, and mercury and selenium analyses of rinsate samples. All required analytical laboratory reports and documentation, including raw data, must be sequentially paginated and clearly labeled with the laboratory name, and associated sample numbers. Any electronic media submitted must be similarly labeled. The sample preparation laboratory and analytical laboratories contracted for homogeneity testing and rinsate analyses will adhere to a comprehensive data management plan that is consistent with the principles set forth in Good Automated Laboratory Practices, EPA Office of Administration and Resources Management (USEPA 1995) or with commonly employed data management procedures approved by the National Environmental Laboratory Accreditation Conference (NELAC).

Data Retention

All computer files associated with the Fish Plug Evaluation Study are stored in a project subdirectory by Tetra Tech, and are copied to network storage for archive for the 5 years subsequent to project completion (unless otherwise directed by the EPA Project Manager).

C. ASSESSMENT AND OVERSIGHT

C1. Assessments and Response Actions

C1.1 Fish Sample Collection

Assessment activities and corrective response actions have been identified to ensure that sample collection activities are conducted as prescribed and that the measurement quality objectives and data quality objectives established by EPA are met. The QA program under which this project is operating includes independent checks of the data obtained from sampling activities and may include performance and system audits. Either type of audit could indicate the need for corrective action. The essential steps in the program are as follows:

- identify and define the problem,
- assign responsibility for investigating the problem,
- investigate and determine the cause of the problem,
- assign and accept responsibility for implementing appropriate corrective action,
- establish effectiveness of and implement the corrective action, and
- verify that the corrective action has eliminated the problem.

Immediate corrective actions can be part of normal operating procedures and, if needed, must be noted on the Fish Plug Evaluation Study Sample Collection Forms. The most common corrective action of this type is the resolution of sampling location, fish specimen, or field-extracted fillet plug information. Each Fish Plug Evaluation Study Sample Collection Form describes the sampling location, species, specimen length, and field-extracted fillet plug samples. The sample information is evaluated by the EPA Project Manager, who decides whether it is suitable for inclusion and analysis. After the EPA Project Manager and the Tetra Tech Project Leader evaluate and reach a decision regarding sample details and resolution elements, approvals or resolutions are recorded on the Fish Plug Evaluation Study Sample Collection Form and initialed by the Tetra Tech Project Leader.

Communication and oversight proceeds from field sampling team leaders (e.g., the senior fisheries biologist) to the Tetra Tech Project Leader. The Tetra Tech Project Leader oversees the review of all Fish Plug Evaluation Study Sample Collection Forms upon receipt, and communicates the status of the sampling activities to the EPA Project Manager on a weekly basis (at a minimum). The Tetra Tech Project Leader will immediately consult with the Tetra Tech QA Officer and EPA Project Manager regarding any difficulties encountered during sample collection activities. The Tetra Tech QA Officer initiates the corrective action system described above, documenting the nature of the problem in a system audit report and ensuring that the recommended corrective action is carried out.

The EPA Project Manager and/or the Tetra Tech QA Officer will work with the Tetra Tech Project Leader to determine the best way to rectify the problem and obtain accurate and useable data. When corrective actions have been taken and a sufficient time period has elapsed that allows a response, the response is compared with project goals by the EPA Project Manager. The Tetra Tech QA Officer verifies that the corrective action has been appropriately addressed to eliminate the problem. The EPA Project Manager may request to stop work on the project if problems affecting data quality are identified that will require extensive effort to resolve. The EPA Project Manager will consult with the EPA QA Officer regarding any and all corrective actions and with the Contracting Officer about requests for stop work orders.

C1.2 Fish Sample Preparation

The sample preparation laboratory supporting this study and the analytical laboratories responsible for lipid testing and rinsate analyses each have a comprehensive QA program in place and operating at all times. In performing sample preparation and analysis work for this study, each laboratory shall adhere to the requirements of those respective QA programs. Copies of those plans are maintained on file at Tetra Tech.

If any technical problems are encountered during operations at the fish sample preparation laboratory, the Tetra Tech Project Leader will consult with the Tetra Tech Laboratory Manager and the EPA Project Manager to identify corrective actions. The Tetra Tech Project Leader is responsible for ensuring that the corrective actions are successfully implemented. Section B5 of this QAPP identifies corrective actions for any lipid, mercury, or selenium analysis results generated by the analytical laboratory (or laboratories) that do not meet the QC acceptance criteria. The Tetra Tech Project Leader is responsible for ensuring that each analytical laboratory implements the required corrective actions.

C1.3 Performance Audits

Performance audits are qualitative checks on different segments of project activities. For the Fish Plug Evaluation Study, performance audit techniques include checks on sampling equipment prior to going into the field, post-collection review of field measurements, and the use of triplicate lipid analyses of one homogenized sample in every fish sample preparation batch as a check on homogeneity. The Tetra Tech Project Leader is responsible for overseeing work as it is performed and for periodically conducting QC checks during fish sample collection and fillet sample preparation for this project. Results of these checks are reported to the Tetra Tech Quality Assurance Officer and the EPA Project Manager.

C1.4 System Audits

System audits are qualitative reviews of project activities to check that the overall quality program is functioning and that the appropriate QC measures identified in the QAPP are being implemented. If the results of the performance audits described in Section C1.3 indicate problems, the Tetra Tech QA Officer will conduct an internal system audit during the project and report the results to the EPA Project Manager. If QA/QC deficiencies are discovered, additional internal system audits are conducted until the Tetra Tech QA Officer and the EPA Project Manager conclude that overall project quality requirements are being met.

C2. Surveillance

C2.1 Fish Sample Collection

The Tetra Tech fish sampling team leader coordinates with other staff at Tetra Tech regarding fish tissue sample collection efforts and sample deliveries to the Tetra Tech laboratory. Prior to collecting samples in the field, the fish sampling team leader oversees mobilization for all field activities. Once in the field, the sampling team leader oversees or performs the collection of whole fish samples, and ensures that the fish samples are collected at the designated locations, that they are of appropriate species and size, and that all field data (including fish length measurements) are recorded. When samples are transported to the Tetra Tech laboratory, the fish sampling team leader contacts designated laboratory staff to notify them of the upcoming sample deliveries and request that they contact the EPA Project Manager when the samples arrive. Within 24 hours of sample receipt, Tetra Tech notifies the EPA Project Manager of sample condition. If problems with the shipment are noted, Tetra Tech will work with the EPA Project Manager to resolve the problem as quickly as possible to minimize data integrity problems.

C2.2 Fish Sample Preparation

The content of fish sample preparation batches and the process for forming the batches are described in Section B4.1. The fish sample preparation laboratory may not begin processing any samples until this QAPP is approved and the laboratory personnel responsible for fish sample preparation have been trained on the fish sample preparation procedures and requirements described in this QAPP.

The Tetra Tech Project Leader coordinates with the EPA Project Manager regarding fish tissue sample shipments to other laboratories (i.e., the analytical laboratories responsible for mercury analysis and for selenium analysis of fish sample preparation equipment rinsate and blank aqueous QC samples and for lipid analysis of homogenized fillet tissue samples from all mercury phase and selenium phase fish samples, including triplicate lipid analysis of one fish sample per fish sample preparation batch for homogeneity testing) once analysis contracts are in place. Tetra Tech communicates periodically with laboratory staff by telephone or email to monitor the progress of lipid, mercury, and selenium analyses. If technical problems are encountered during sample preparation or during lipid, mercury, or selenium and percent solids analyses, the Tetra Tech Project Leader will identify a technical expert within Tetra Tech to assist in resolving the problem, and work with the EPA Project Manager to identify and implement a solution to the problem. The fish sample preparation laboratory is permitted to work 5 batches ahead of the delivery and review of batch-specific mercury phase QC results that indicate if the homogenization and equipment cleaning procedures for each fish sample preparation batch are adequate, and 3 batches ahead if QC results are determined to be in compliance during the selenium phase.

If the sample preparation or analytical laboratories fail to deliver QC data on time, or if an analytical laboratory notifies Tetra Tech of anticipated reporting or sample processing delays, Tetra Tech notifies the EPA Project Manager of the situation. To the extent possible, Tetra Tech

will adjust schedules and shift resources as necessary to minimize the impact of laboratory delays on EPA schedules. Tetra Tech will immediately notify the EPA Project Manager of any laboratory delays that are anticipated to impact EPA schedules.

C3. Reports to Management

Upon completion of weekly fish sampling and sample preparation activities, the Tetra Tech Project Leader provides the EPA Project Manager with reports of fish sampling team and sample preparation laboratory progress for the preceding week when these activities are occurring. These weekly progress reports include specific details about the fish sample collection and fillet sample preparation activities, and note any concerns about sample quality and resolution of those concerns. Following completion of fish sampling and fillet sample preparation activities, Tetra Tech prepares a fish collection effort summary (which details all sampling participants, sampling locations, and specimens collected) and a sample preparation summary (which lists all samples processed and identifies all fillet tissue aliquots prepared) for review by the EPA Project Manager.

D. DATA VALIDATION AND USABILITY

D1. Data Review, Verification, and Validation

The processes for data review, verification, and validation provide an approach for standardized data quality assessment. These processes are also important for determining the usability and limitations of the fish sample collection and preparation data generated by the Fish Plug Evaluation Study. Processes for each step in the Fish Plug Evaluation Study data quality assessment are described below.

D1.1 Data Review

Fish Sample Collection

The Tetra Tech fish sampling team leader reviews data entries in the Fish Plug Evaluation Study Sample Collection Form, the Chain-of-Custody Record Form and individual fish sample labels for completeness, correctness, and consistency among the fish sampling records. Any errors or omissions identified during this review are corrected by the team member who initially made the data entries. The Tetra Tech fish sampling team leader is responsible for ensuring that all errors or omissions are addressed in the fish sampling records before the fish samples are delivered to the fish sample preparation laboratory.

Analysis of Lipid and Fish Sample Preparation QC Samples

The Laboratory Managers at each analytical laboratory review all laboratory results and calculations prior to submission of a data package. Any errors identified during this peer review are returned to the analyst for correction. Following correction of the errors, each Laboratory Manager verifies that the final data package is complete and compliant with the contract, signs each data submission to certify that the package was reviewed and determined to be in

compliance with the terms and conditions of the contract, and submits the data package to the Tetra Tech Project Leader.

D1.2 Data Verification

The basic goal of data verification is to ensure that project participants know what data were produced, if these data are complete, if the data are contractually compliant, and if the data meet the objectives of the study and the QA requirements described in this QAPP.

Fish Sample Collection

Tetra Tech staff independent of fish sampling teams verify fish sample collection data reviewed and submitted by each fish sampling team leader. This involves verifying that all data entries in the Fish Plug Evaluation Study Sample Collection Form, the Chain-of-Custody Record Form, and the individual fish sample labels are complete, correct, and consistent among the fish sampling records. The data verifier reports any discrepancies identified during this process to the Tetra Tech Project Leader. The Tetra Tech Project Leader is responsible for reconciling any discrepancies reported during data verification with the appropriate associated field personnel and for notifying the data verifier about the resolution of these discrepancies. The data verifier is responsible for documenting resolution of these data entry discrepancies.

Analysis of Lipid and Fish Sample Preparation QC Samples

The Tetra Tech Laboratory Manager conducts initial reviews of the fish sample preparation QC sample results and single lipid analysis results associated with each of the 30 fish sample preparation batches for the mercury phase and the 6 sample preparation batches for the selenium phase to verify the completeness and accuracy of these data and their compliance with QC acceptance criteria in Section B5 of this QAPP. Verification of these results involves review of data for percent lipid measurements (including the 30 triplicate lipid analysis results for the homogeneity testing of one fish sample per fish sample preparation batch for mercury and the 6 triplicate lipid analysis results for one fish sample per selenium preparation batch, along with lipid analysis results for the remaining 30 individual mercury fish samples and the remaining 24 selenium fish samples) and review of sample processing equipment rinsate and corresponding blank QC samples. The Tetra Tech Project Leader verifies the summary level results for these QC samples and remaining lipid samples, determines if they meet the project objectives in this QAPP, and reports the verification findings to the EPA Project Manager. The CSRA analytical chemist supporting the Fish Plug Evaluation Study will conduct an independent review of analytical results for lipids and for fish sample preparation QC samples and report the verification findings to the EPA Project Manager. The EPA Project Manager will work with the Tetra Tech Project Leader and the CSRA analytical chemist to resolve any differences in their respective verification findings.

D1.3 Data Validation

Data validation is the process of evaluating the quality of the results relative to their intended use. Data need not be "perfect" to be usable for a particular project, and the validation process is designed to identify data quality issues uncovered during the certification process that may affect

the intended use. One goal of validation is to answer the "So what?" question with regard to any data quality issues.

Fish Sample Collection

Evaluating the quality of fish sample collection results involves comparing these results to the fish sampling requirements described in Section B4 of this QAPP. These requirements include collecting fish samples from specific waterbodies and obtaining particular fish species and numbers of fish to meet study objectives.

Fish Sample Preparation

The data validation process for fillet tissue sample preparation is more limited than the process applied during validation of analytical data from analysis of fillet tissue samples for the study-specific target chemicals. It focuses on evaluating the clarity and accuracy of required information in the fish sample preparation weekly progress reports (e.g., percentage of total fillet mass compared to the total body mass, tissue mass requirements for specified tissue aliquots, etc.).

D2. Verification and Validation Methods

D2.1 Verification Methods

Fish Sample Collection Data

The initial step in the process for data verification involves Tetra Tech staff independent of fish sampling operations conducting reviews of all data related to fish sample collection as a means of identifying any discrepancies among sampling data and related information entered in Sample Collection Forms, Chain-of-Custody Record Forms, and fish sample labels. Results from each review are documented in a series data verification forms and compiled in a data review notebook that is submitted to the EPA Project Manager after the end of the fish sampling season (i.e., 8 weeks after completion of the final fish sampling trip). This notebook includes each Sample Collection Form as well as detailed results of the QC review of the fish sampling data and sample description information that are recorded on standard forms (i.e., the Data Review Form and the Sample Description Review Form). Any discrepancies among the fish sampling records and resolution of these discrepancies are reported to the EPA Project Manager.

Lipid Data and Fish Sample Preparation QC Sample Data

The first stage in the data verification process involves the Tetra Tech Laboratory Manager performing a "Completeness Check" in which all elements in each analytical laboratory submission are evaluated to verify that results for all specified samples are provided, that data are reported in the correct format, and that all relevant information, such as preparation and analysis logs, are included in the data package. Corrective action procedures will be initiated if deficiencies are noted. The Tetra Tech Lab Manager will transmit the analytical laboratory submission to the EPA Project Manager and the CSRA data review chemist.

The second stage of the data verification process focuses on an "Instrument Performance Check" in which the CSRA data review chemist verifies that calibrations, calibration verifications, standards, and calibration blanks, as they apply to either lipid analysis or analysis of fish sample preparation QC samples, were analyzed at the appropriate frequency and met method or study performance specifications. If errors are noted at this stage, CSRA will identify corrective action procedures for Tetra Tech to initiate with the analytical laboratory immediately.

Stage three of the data verification process focuses on a "Laboratory Performance Check" in which the CSRA data review chemist verifies that the laboratory correctly performed the required analytical procedures and was able to demonstrate a high level of precision and accuracy. This stage includes evaluation of QC elements such as laboratory control samples, method blanks, matrix spike samples and/or reference samples, as they apply to either analysis of lipid samples or fish sample preparation QC samples. CSRA will provide corrective action procedures for Tetra Tech to initiate with the analytical laboratory to resolve any deficiencies identified.

D3. Reconciliation with User Requirements

Fish Sample Collection Data

As soon as possible following completion of fish sampling operations, The Tetra Tech Project Leader assesses fish sample collection data for completeness, precision, and representativeness by comparing these data with the criteria discussed for each of these measures in Section A7 of this QAPP. This represents the final determination of whether the fish samples collected for the Fish Plug Evaluation Study are of the correct type, quantity, and quality to support their intended use for this study. The Tetra Tech Project Leader will report any problems encountered in meeting the performance criteria (or uncertainties and limitations in the use of the data) to the EPA Project Manager, and work with the EPA Project Manager to reconcile the problems, if possible.

Lipid Data and Fish Sample Preparation QC Sample Data

The QC results for lipids from the homogeneity testing and for the mercury or selenium rinsate analyses from homogenization of fillet tissue samples for each fish sample preparation batch are assessed by the CSRA data review chemist against the QC acceptance criteria in Section B5 of this QAPP. Although the sample preparation laboratory will be permitted to work 5 batches ahead of the delivery of the batch-specific QC results for the mercury phase and 3 batches ahead for the selenium phase, the Tetra Tech Project Leader will track laboratory performance, notify the EPA Project Manager of any issues, initiate corrective actions, and track progress by the fish sample preparation laboratory.

References

APHA. 2005. Standard Method 2450G. Total, Fixed, and Volatile Solids in Solids and Semisolid Samples. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, 21st edition, Washington, DC.

Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911-917.

USEPA. 1994a. Method 245.1: Determination of Mercury in Water by Cold Vapor Atomic Absorption Spectrometry (CVAA).

USEPA. 1994b. Method 200.8. Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Mass Spectrometry, Revision 5.4. U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring Systems Laboratory, Cincinnati, OH.

USEPA. 1995. Good Automated Laboratory Practices. EPA Manual 2185. U.S. Environmental Protection Agency, Office of Administration and Resources Management, Washington, DC, August 1995.

USEPA. 2000. Guidance for assessing chemical contaminant data for use in fish advisories, Vol 1. Fish sampling and analysis. EPA-823-B-00-007. United States Environmental Protection Agency, Office of Water, Washington, DC.

USEPA. 2001. EPA Requirements for Quality Assurance Project Plans. EPA QA/R-5. U.S. Environmental Protection Agency, Office of Environmental Information, Washington, DC. EPA/240/B-01/003.

USEPA. 2018. Quality Assurance Project Plan for Analysis of Fish Fillet Samples for the Fish Plug Evaluation Study, Revision 1. U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Washington, DC.

Appendix A

Fish Plug Evaluation Study Sample Collection Procedures

SCOPE AND APPLICABILITY:
These sample collection procedures must be followed by all field sample collection teams involved with the EPA's Fish Plug Evaluation Study. Adherence to these procedures ensures that field sampling activities are performed the same way every time, i.e., are standardized, for all field personnel. Whole fish and field-extracted fillet plug sampling procedures are presented below as sequential steps, and they include specific equipment, materials, and methods required to perform field sampling activities only.

RESPONSIBILITY AND PERSONNEL QUALIFICATIONS:
These procedures must be used by all field sample collection teams that have been authorized by the EPA Project Manager to collect fish for the Fish Plug Evaluation Study.

PRECAUTIONS:
Field sample collection teams must follow usual safety precautions for working in the field. Boats and/or electrofishing equipment will only be operated by qualified, experienced operators trained in their proper use. Each vessel must be equipped with the appropriate Coast Guard-required safety equipment (including personal flotation devices for each field team member). If electrofishing equipment is used for sample collection, each team member must be insulated from the water, boat, and electrodes via rubber boots and gloves.

EQUIPMENT/MATERIALS:
Exhibit 1 lists the equipment and supplies necessary for field teams to collect whole fish samples and the field-extracted fillet plug samples. This list provides information to ensure that field crews bring the required equipment to each sampling site.

Exhibit 1. Equipment and Supplies

For **collecting** whole fish and field-extracted fillet plug samples
• Electrofishing equipment (including variable voltage pulsator unit, wiring cables, generator, electrodes, dip nets, protective gloves, boots, and necessary safety equipment) for river sampling • Hook and line for Great Lake sampling • Scientific collection permits and/or fishing licenses • Sampling vessel (including boat, motor, trailer, oars, gas, and all required safety equipment) • Coast Guard approved personal flotation devices • Cellphone or vessel-based radio for emergency communications • Global Positioning System (GPS) unit • Livewell and/or buckets • Measuring board (millimeter scale) • Clean nitrile gloves • Plastic cable ties • Aluminum foil (solvent-rinsed and baked) • Heavy-duty food grade polyethylene tubing • Small plastic bags and bubble wrap bags • 8-mm disposable biopsy punches (Acuderm brand Acu-punch or equivalent) • Sterile disposable scalpels • Sterile 20-mL glass scintillation vials • Laboratory pipette bulbs • Knife or scissors • Coolers • Dry ice
For **documenting** whole fish and field-extracted fillet plug samples
• Fish Plug Evaluation Study Sample Collection Forms • Whole Fish Labels • Field Plug Labels • Clipboard • Pens (black ink) for recording data on sample collection forms • Fine-tipped indelible markers for filling out sample labels • Clear tape strips for covering labels
For **shipping** whole fish and field-extracted fillet plug samples
• Preaddressed FedEx airbills • Custody Seals • Chain-of-Custody Forms • Packing/strapping tape • Dry Ice • Coolers

PROCEDURES:

1. Identify the target sampling location approved by the EPA Fish Plug Evaluation Study Project Manager before proceeding with sample collection. Sampling sites in the Great Lakes are in Lake Erie, Lake Michigan, and Lake Ontario. River sites are in the Anacostia River, Potomac River, and St. Lawrence River. Locate each sampling site on topographic maps, GPS, or equivalent maps or mapping software.
2. Conduct site reconnaissance to identify the best ports, launches, or access points to allow sampling of the site and to optimize the opportunity to collect the desired target numbers and species of fish.
3. Assemble an array of active gear types (e.g., electrofishers and hook-and-line equipment) to ensure the collection of the desired target numbers and species of fish. Selection of the most appropriate gear type(s) will be at the discretion of the experienced on-site fisheries biologist. Sampling teams must be qualified, experienced, and trained on the safe and effective use of each gear type selected.
4. Identify fish to species as soon as they have been obtained via active collection methods, and select target specimens (see Exhibit 2 for target fish species). Use a GPS unit to identify the specific sampling coordinates (i.e., latitude and longitude) for each target fish specimen retained for analysis. Record sampling coordinates on the Fish Plug Evaluation Study Sample Collection Form (Exhibit 3 for the mercury phase and Exhibit 4 for the selenium phase).
5. Put on clean nitrile gloves immediately before the fish sample handling process and do not handle any food, drink, sunscreen, or insect repellant before handling the fish.
6. Rinse potential target species/individuals in ambient water to remove any foreign material from the external surface and place them in clean holding containers (e.g., livewells, buckets). Target species are listed in **Exhibit 2** below. Return non-target fishes or small specimens to the source waterbody.

Exhibit 2. Target Fish Species for the Fish Plug Evaluation Study

Location		Common Name	Scientific Name	# of Fish (Hg Phase)	# of Fish (Se Phase)
Great Lakes	Rivers*				
Lake Erie		Walleye	*Sander vitreus*	10	5
Lake Michigan		Lake trout	*Salvelinus namaycush*	10	5
Lake Ontario		Chinook salmon	*Oncorhynchus tshawytscha*	10	5
	Anacostia River	Blue catfish	*Ictalurus furcatus*	10	5
	Potomac River	Largemouth bass	*Micropterus salmoides*	10	5
	St. Lawrence River	Smallmouth bass	*Micropterus dolomieu*	10	5

7. Retain 10 individuals of a target species from the sampling site for the mercury phase of the study and 5 individuals of a target species from the sampling site for the selenium phase of the study. The fish should be of adequate size to provide all plug and fillet samples needed for the Fish Plug Evaluation Study. Select fish based on the following criteria:

    - up to 10 adult individuals of the target species are available from the sampling site (10 fish are required for the mercury phase and 5 fish are required for the selenium phase),
    - all specimens satisfy legal requirements of harvestable size for the sampled river or Great Lake, or at least are of a size large enough to provide all plug and fillet samples needed for the Fish Plug Evaluation Study if no legal harvest requirements are in effect.

    Accurate taxonomic identification (to species) is essential in assuring that the correct target species have been collected and in defining the fish species to be submitted for analysis.

8. Remove each fish retained for analysis from the clean holding container(s) (e.g., livewell) while wearing clean nitrile gloves. Dispatch each fish using a clean wooden bat (or equivalent wooden device).

9. Measure each target fish specimen to determine total body length. Measure total length of the specimen in millimeters, from the anterior-most part of the fish to the tip of the longest caudal fin ray (when the lobes of the caudal fin are depressed dorsoventrally).

10. Note any anomalies (e.g., lesions, cuts, sores, tumors, fin erosion) observed on the fish.

11. Assign the unique eight-character sample ID number to each individual fish sample as directed on the Fish Plug Evaluation Study Sample Collection Form (Exhibit 3 for the mercury phase and Exhibit 4 for the selenium phase).

12. Record sample ID, date, time, species (common name), specimen length, and location on the Fish Plug Evaluation Study Sample Collection Form (Exhibit 3 for the mercury phase and Exhibit 4 for the selenium phase). Make sure that the sample ID numbers and specimen numbers/lengths that are recorded on the sample collection form match those on the corresponding labels for each whole fish sample and field-extracted plug sample.

13. Prepare a Field Plug Label (Exhibit 5 for the mercury phase plug samples, and Exhibits 6 and 7 for the selenium and percent solids (identified as percent moisture for data reporting purposes) plug samples, respectively, collected during the selenium phase) for the sample, ensuring that the label information matches the information recorded on the Fish Plug Evaluation Study Sample Collection Form. Affix the label to a sterile 20-milliliter scintillation vial and cover it with clear tape.

14. Put on clean nitrile gloves before beginning the field plug extraction process.

15. Remove scales (using a sterile disposable scalpel) from a small area on the left side dorsal area of the fish between the dorsal fin and the lateral line.

16. Insert the 8-millimeter biopsy punch into the dorsal muscle of the fish through the scale-free area. Insert the punch with a slight twisting motion, cutting the skin and muscle tissue. Once full depth penetration of the punch is achieved, bend or tilt the punch to break off the end of the tissue plug. Remove the biopsy punch, taking care to ensure the plug remains in the punch. The full depth of the punch must be filled with muscle tissue to ensure collection of sufficient plug biomass for mercury analysis or for selenium and percent solids analyses.

17. Place a pipette bulb on the handle end of the biopsy punch and give a quick squeeze to use air pressure to force the fillet plug into a sterile 20-milliliter scintillation vial.

18. Repeat steps 15-17 and place a second plug into the same sterile 20-milliliter scintillation vial as the first plug. Each mercury and selenium plug sample extracted during the two study phases contains two plugs. In addition, during the selenium phase, repeat steps 15-17 to collect a single-plug sample for percent solids analysis (one plug per sample). Seal the cap on the 20-milliliter scintillation vial.

19. Place the 20-milliliter scintillation vial into a small bubble wrap bag and a plastic bag before placing the sample immediately on dry ice for storage until it is transported or shipped to the fish sample preparation laboratory at the Tetra Tech facility in Owings Mills, MD.

20. ***For the mercury phase --*** Repeat Steps 13-20 on the same fish 4 additional times (for a total of 5 field-extracted fillet plug samples). Place each sample (2 plugs per sample) in a separate 20-milliliter scintillation vial. ***For the selenium phase --*** Repeat steps 13-20 on the same fish 3 additional times (for a total of 4 field-extracted fillet plug samples). Place each sample (2 plugs per sample) in a separate 20-milliliter scintillation vial. In addition, repeat steps 13-20 on the same fish 4 more times to collect a total of 4 single-plug fillet samples for percent solids analysis. Place each percent solids sample (1 plug per sample) in a separate 20-milliliter scintillation vial.

21. Dispose of the nitrile gloves, scalpel and biopsy punch.

22. Pack frozen field-extracted plug samples in coolers on dry ice and hand deliver or ship them to Tetra Tech in Owings Mills, MD via priority overnight delivery service within one week of collection. Fish plug samples may be batch shipped (a maximum of 20 plug samples per cooler) but samples must be kept frozen at $\leq 20^{\circ}C$ until hand delivered or shipped on dry ice. Refer to Steps 27-29 for shipping options and instructions.

23. Put on clean nitrile gloves after field plug extraction is completed. Wrap each whole fish in heavy-duty aluminum foil with the dull side in (foil provided by EPA as solvent-rinsed, oven-baked sheets).

24. Prepare a Whole Fish Label (Exhibit 8) for each fish, ensuring that the label information matches the information recorded on the Fish Plug Evaluation Study Sample Collection Form.

25. Cut a length of food grade tubing that is long enough to contain the individual fish and to allow extra length of tubing. Seal each end of the tubing with a plastic cable tie. Attach the fish sample label to the outside of the food grade tubing with clear tape and secure the label by taping around the entire fish (so that tape sticks to tape).

26. Place each packaged whole fish sample immediately on dry ice for transport or shipment. If samples will be held on board a vessel or in a vehicle (temporarily) before being frozen for transport or shipment, the wrapped whole fish samples can be held on wet ice in coolers during transport to an interim location for freezing.

27. Samples may be stored temporarily on dry ice (replenishing the dry ice daily) with the option of:

    - holding samples temporarily on wet ice and freezing the samples within 12 hours of collection at ≤-20˚C in a portable freezer, and storing the frozen samples in the freezer before (and during) hand delivery to the Tetra Tech laboratory (Owings Mills, MD), or

    - shipping the samples packed on dry ice in sufficient quantities to keep samples frozen for up to 48 hours (minimum of 50 pounds for whole fish shipments and minimum of 30 pounds for plug sample shipments), via priority overnight air delivery service (e.g., FedEx), so that they arrive at the fish sample preparation laboratory (Tetra, Tech, Owings Mills, MD) within less than 24 hours from the time of sample collection, or

    - storing the frozen samples until shipment within 1 week of sample collection (frozen whole fish samples will subsequently be packed on 50 pounds of dry ice and frozen field plug samples will subsequently be packed on 30 pounds of dry ice, then shipped to the Tetra Tech fish sample preparation laboratory in Owings Mills, MD via priority overnight air delivery service).

28. Complete a Chain-of-Custody Record Form for each whole fish and field plug sample cooler (Exhibit 9). All entries must be in black ink and coincide with fish sample information on the Fish Plug Evaluation Study Sample Collection Forms and the corresponding Whole Fish Labels or Field Plug Labels. Retain one copy of the Chain-of-Custody Form, place and seal all other copies in a waterproof bag, and enclose the sealed form in the shipping cooler. Pack each cooler (completely) with 50 pounds of dry ice for whole fish samples or 30 pounds of dry ice for field plug samples, secure each cooler with packaging tape, and seal it (e.g., across the latch of the ice chest) with a Custody Seal (Exhibit 10). Include the signature of the sampler on each Custody Seal and the date and time the cooler was sealed (in black ink).

29. Transport or ship coolers containing whole fish samples (for shipping, maximum of 5 fish per cooler during both the phases of the study) and field plug samples (for shipping, maximum of 20 plug samples per cooler during both phases of the study) to the Tetra Tech fish sample preparation laboratory. **Samples must be shipped on Monday through Wednesday to allow sufficient delivery time prior to the weekend.**

Exhibit 3. Fish Plug Evaluation Study Mercury Phase Sample Collection Form

Fish Plug Evaluation Study Sample Collection Form

Sample Identification: ____ ____ ____ ____ ____ ____ ____ ____

Sample ID Key:

Waterbody type	Site identifier	Species abbreviation	Specimen number
GL = Great Lakes	ER = Erie MI = Michigan ON = Ontario	SA = Salmon LT = Lake trout WA = Walleye	01 to 10
RV = River	AN = Anacostia PT = Potomac SL = St. Lawrence	BC = Blue catfish LB = Largemouth bass SB = Smallmouth bass	01 to 10

Sampling Date: ____/____/_____ Sampling Time: _____

Collector(s) Name(s): _____

Collection Method (circle one): Hook & Line / Electrofishing / Other _____

Site Location

River/Great Lakes Name (Location): _____

Location Description: _____

Latitude: _____ Longitude: _____
(Degrees, Minutes, Seconds)

Latitude: _____ Longitude: _____
(Decimal Degrees)*

Sample Description

Fish Species (Common Name): _____ Fish Length (mm): _____

Whole Fish Sample ID (See Sample ID Key above): _____

Field Fish Plug Sample ID (See Sample ID Key above)	**Field Plug Sample Taken** (2 plugs each)
_____**FP1**	YES/NO
_____**FP2**	YES/NO
_____**FP3**	YES/NO
_____**FP4**	YES/NO
_____**FP5**	YES/NO

Additional Comments: _____

_____

_____

***The conversion to decimal degrees can be done in the lab or decimal degrees can be recorded directly in the field to 5 decimal degrees.**

Exhibit 4. Fish Plug Evaluation Study Selenium Phase Sample Collection Form

Fish Plug Evaluation Study Selenium Phase Sample Collection Form

Sample Identification: ____ ____ ____ ____ ____ ____ ____ ____

Sample ID Key:

Waterbody type	Site identifier	Species abbreviation	Specimen number
GL = Great Lakes	ER = Erie MI = Michigan ON = Ontario	SA = Salmon LT = Lake trout WA = Walleye	01 to 05
RV = River	AN = Anacostia PT = Potomac SL = St. Lawrence	BC = Blue catfish LB = Largemouth bass SB = Smallmouth bass	01 to 05

Sampling Date: ____/____/_____ Sampling Time: _____

Collector(s) Name(s): _____

Collection Method (circle one): Hook & Line / Electrofishing / Other _____

Site Location

River/Great Lakes Name (Location): _____

Location Description: _____

Latitude: _____ Longitude: _____
(Decimal Degrees)*

Sample Description

Fish Species (Common Name): _____ Fish Length (mm): _____

Whole Fish Sample ID (See Sample ID Key above): _____

Field Fish Plug Sample ID (See Sample ID Key above)	**Field Plug Sample Taken** (2 plugs each)	**% Moisture Plug Sample ID** (See Sample ID Key above)	**% Moisture Plug Taken** (1 plug each)
_____**FP1**	YES/NO	_____**FP1%M**	YES/NO
_____**FP2**	YES/NO	_____**FP2%M**	YES/NO
_____**FP3**	YES/NO	_____**FP3%M**	YES/NO
_____**FP4**	YES/NO	_____**FP4%M**	YES/NO

Additional Comments: _____

_____

_____

_____

*** Record decimal degrees directly in the field to 5 decimal degrees.**

Exhibit 5. Mercury Phase Field Plug Label

FPES Field Plug Label
Sample ID: _____
Location: _____
Date: ___/___/_____ Time: _____
Species: _____
Fish Length (mm): _____ Est. wt (g): _____

Exhibit 6. Selenium Phase Field Plug Label

FPES Selenium Phase Field Plug Label
Sample ID: _____
Location: _____
Date: ___/___/_____ Time: _____
Species: _____
Fish Length (mm): _____ # of Plugs: _____

Exhibit 7. Selenium Phase Percent Moisture Label

FPES Percent Moisture Field Plug Label
Sample ID: _____
Location: _____
Date: ___/___/_____ Time: _____
Species: _____
Fish Length (mm): _____ # of Plugs: _____

Exhibit 8. Whole Fish Label

FPES Whole Fish Label
Location: _____ Date: ____/____/_____
Sample ID: _____ Time: _____
Species: _____
Length (mm): _____

Exhibit 9. Chain-of-Custody Record Form

Tetra Tech, Inc. | Biological Research Facility ***CHAIN-OF-CUSTODY RECORD***

Project Manager or Client Contact:	Preservative	Number of	Type of Analyses Requested								**Shaded area for Tt use only:**
Address/Phone:											Sample check-in: DO _____ pH _____ Cond/Salinity _____ Chlorine _____ Appearance _____
Collector Name/Phone:											
Project Number: Project Name:											
Page of Sample Location:											
Date / Time / Sample Identification/Station											Collection Method / Log Number

Sampled by: *(signature)*	Date/Time:	Relinquished by: *(signature)*	Date/Time:	Received by: *(signature)*	Date/Time:
Relinquished by: *(signature)*	Date/Time:	Received by: *(signature)*	Date/Time:	Received by: *(signature)*	Date/Time:

FORM DISTRIBUTION: WHITE – Tt BRF YELLOW- Report PINK - Sampler

Exhibit 10. Custody Seal

CUSTODY SEAL

Signature_____

Date and Time_____

Appendix B

Fish Plug Evaluation Study Mercury Phase Fillet Tissue Preparation, Homogenization, and Distribution Procedures

Appendix B

Fish Plug Evaluation Study Mercury Phase
Fillet Tissue Preparation, Homogenization, and Distribution Procedures

I. PURPOSE

This document describes the procedures that the fish sample preparation laboratory follows when preparing fish fillet tissue samples for the mercury phase of EPA's Fish Plug Evaluation Study (FPES) under contract to EPA. Adherence to these procedures ensures that fish tissue preparation activities at the Tetra Tech laboratory in Owings Mills, MD are performed consistently across all study samples and in a manner consistent with previous EPA fish tissue studies. The effort is divided into two primary components:

- Fish fillet tissue processing and distribution procedures, including quality control steps, for the 30 fish sample preparation batches.
- Analyses of rinsate and blank samples for mercury (30 pairs corresponding to each of the fish sample preparation batches).

Each of these components is described in detail below.

II. FISH FILLET TISSUE PROCESSING AND DISTRIBUTION PROCEDURES

The procedures for processing and distributing FPES fillet tissue samples are described below. The process description is organized into the following components, including the quality control (QC) procedures:

A. Sample Receipt and Storage
B. Sample Handling
C. Filleting and Homogenization Procedures, Including Removal of Plug Samples for Mercury Analysis
D. Aliquoting and Distribution Procedures
E. Equipment Cleaning between Fish Samples
F. Lipid Determination on Every Homogenized Fillet Sample
G. Quality Control (QC) Procedures
H. Reporting Requirements
I. Shipping Samples

The individual steps in the overall process are presented as a series of numbered steps across the nine components listed above.

Fillet Tissue Processing Definitions

- **Whole Fish Sample:** A whole fish sample for the Fish Plug Evaluation Study consists of the single fish sample collected for each target species at the 6 designated sampling locations (3 Great Lakes, including Lake Erie, Lake Michigan, and Lake Ontario, and 3 mid-Atlantic rivers, including the Anacostia River, Potomac River, and St. Lawrence River). For the mercury phase of the study, 10 individual whole fish samples are collected from each sampling location as follows: Lake Erie, walleye; Lake Michigan, lake trout; Lake Ontario, Chinook salmon; Anacostia River, blue catfish; Potomac River, largemouth bass; and St. Lawrence River, smallmouth bass. Field crews collect a total of 60 whole fish samples during the mercury phase

of the study and transport or ship them to the fish sample preparation laboratory (Tetra Tech laboratory, Owings Mills, MD).

- **Fillet plug sample:** A fillet plug sample consists of 2 plugs of fillet tissue that are removed from a whole fish sample using an 8-mm biopsy punch. For the mercury phase of the study, 5 fillet plug samples are removed from a whole fish sample in the field and another 5 fillet plug samples are removed from that whole fish sample in the fish sample preparation laboratory. Each plug sample should contain 1.0 to 1.5 grams of fillet tissue. The mercury phase of the Fish Plug Evaluation Study will generate 300 field-extracted fillet plug samples and 300 lab-extracted fillet plug samples for mercury analysis.

- **Fish sample preparation batch:** Each fish sample preparation batch consists of 2 individual whole fish samples. The 60 whole fish samples are assigned to 30 fish sample preparation batches (based on chronological order that fish samples are collected, beginning with the oldest samples first). The mercury phase fish processing instructions are the same for each of the 30 fish sample preparation batches. Processing the 2 whole fish samples in the fish sample preparation batch involves removal of 5 replicate fillet plug samples and preparation of 5 replicate homogenized fillet tissue samples from each of the fish in the batch. Each fish sample preparation batch produces a total of 10 fillet plug samples and 10 homogenized fillet tissue samples for mercury analysis.

- **Mercury analysis batch:** A mercury analysis batch consists of the 20 fillet tissue samples generated during processing of a fish sample preparation batch. These 20 fillet tissue samples include the 10 lab-extracted fillet plug samples (5 replicate samples per fish) and the corresponding 10 homogenized fillet tissue samples (5 replicates per fish) from the 2 whole fish samples in a fish sample preparation batch.

II.A Sample Receipt and Storage

Tetra Tech field crews collected the fish samples for the Fish Plug Evaluation Study during August and September 2017. Sixty (60) fish were collected for the mercury phase of this study, consisting of 10 individual fish samples for each of six species (three Great Lakes species and three mid-Atlantic river species).

Samples were hand-delivered to the sample preparation laboratory for storage and processing. The sample preparation laboratory must have sufficient freezer space to store **up to 60 unprocessed fish samples** at a temperature of less than or equal to -20 °C from the time of receipt until completion of sample processing and sufficient freezer space to store **fillet plug samples and homogenized fillet tissue aliquots from up to 60 processed samples** (e.g., up to 960 jars) prior to distribution.

1. Although samples are delivered frozen, on dry ice, they must be inspected promptly on receipt. As samples are received, the sample custodian must:

   - Check that each shipping container has arrived undamaged and verify that samples are still frozen and in good condition.
   - Check the temperature of one of the samples in the cooler using a thermometer that reads to at least -20 °C, or an infra-red (IR) temperature "gun," and record the reading.
   - Verify that all associated paperwork is complete, legible, and accurate.
   - Compare the information on the label on each individual fish sample and on each field-extracted fillet plug sample to the sample tracking form to verify that each specimen was included in the shipment and is properly wrapped and labeled.

- Notify the EPA Project Manager of the fact that samples were received on the day of delivery and report any discrepancies in the paperwork identified above.
- Check that the samples were collected from the designated Great Lakes or river sites and notify the EPA Project Manager of any changes in sampling locations.
- Transfer the samples to the freezer for long-term storage.

2. Notify the EPA Project Manager immediately about any problems encountered upon receipt of samples. Problems involving sample integrity, conformity, or inconsistencies for fish samples should be reported to the EPA Project Manager in writing (e.g., by email) within one business day following sample receipt and inspection.

   Following fillet sample processing, the fish sample preparation laboratory must store fillet plug samples and homogenized fillet tissue sample aliquots frozen to less than or equal to -20 °C until they are distributed to the laboratory designated for mercury analysis of FPES fillet samples (ALS Environmental in Kelso, WA).

II.B Sample Handling

The whole fish samples collected for the FPES must remain frozen at less than or equal to -20 °C until the fish sample preparation laboratory receives direction from the EPA Project Manager to begin processing the samples. Samples to be processed must be retrieved from the freezer, with their associated paperwork, and allowed to partially thaw before they can be processed.

3. The fish sample processing instructions are the same for all 30 fish sample preparation batches. Each fish sample preparation batch consists of 2 whole fish samples. Prior to beginning fish sample processing, Tetra Tech prepares an Excel spreadsheet assigning whole fish samples to fish sample preparation batches and submits the spreadsheet for approval by the EPA Project Manager. Processing for a fish sample preparation batch involves the following for each fish in the batch:

   - Removal of 5 replicate fillet plug samples (2 plugs per plug sample)
   - Preparation of 5 replicate homogenized fillet tissue samples.

   Note: Processing a fish sample preparation batch produces a total of 10 fillet plug samples and 10 homogenized fillet tissue samples, which constitutes a mercury analysis batch.

4. When retrieving samples from the freezer, the sample custodian must:

   - Verify that all associated paperwork stored with the samples is complete, legible, and accurate.
   - Compare the information on the label on each individual fish sample to the fish sample preparation batch spreadsheet and notify the EPA Project Manager of any discrepancies between the sample labels and this Excel file. Problems involving sample paperwork, sample integrity, or custody information inconsistencies for all fish samples should be reported to the EPA Project Manager in writing (e.g., by email) within one business day following sample retrieval and inspection. **Do not proceed with sample processing until discrepancies are resolved.**

II.C. Filleting and Homogenization Procedures, Including Removal of Plug Samples for Mercury Analysis

As part of the overall FPES, mercury analyses are performed on three types of fillet tissue samples: field-extracted fillet plug samples, laboratory-extracted fillet plug samples, and aliquots of homogenized fillet tissue samples. Sample processing includes collection of 5 plug samples (each sample consisting of

2 plugs) from each whole fish sample in the fish sample preparation batch, using the procedures described in Steps 10 - 16. Steps 5 - 9 below must be completed before beginning collection of fillet plug samples.

5. Prior to preparing any fillet samples, thoroughly clean utensils and cutting boards using the following series of procedures:

   - Wash with a detergent solution (phosphate- and scent-free) and warm tap water
   - Rinse three times with warm tap water
   - Rinse three times with deionized (DI) water
   - Rinse with acetone
   - Rinse three times with DI water
   - Rinse with (not soak in) 5% nitric acid
   - Rinse three times with DI water

   To control contamination, separate sets of utensils and cutting boards must be used for scaling fish and for filleting fish.

   Note: A new biopsy punch is used for collecting the series of 5 plug samples from each whole fish sample, then discarded. Biopsy punches are **not** subjected to the cleaning procedures above.

6. Put on powder-free nitrile gloves before unpacking an individual fish sample for plug sample collection and for filleting and tissue homogenization. After unwrapping, inspect each fish carefully to verify that it has not been damaged during collection or shipment. If damage (e.g., tearing the skin or puncturing the gut) is observed, document it in the laboratory project log sheet and notify the EPA Project Manager before proceeding further.

7. Weigh each fish to the nearest gram (wet weight) prior to any sample processing. Enter weight information for each individual fish into a laboratory project log sheet. Individual fish sample weights eventually will be transferred to spreadsheets for submission to the EPA Manager.

8. Rinse each fish with deionized water as a precautionary measure to treat for possible contamination from sample handling in the field. Use HDPE wash bottles for rinsing fish and for cleaning homogenization equipment and utensils.

9. Before beginning the scaling process for each fish, put on new powder-free nitrile gloves. (Gloves must be changed *between* individual fish samples, but the same gloves may be used for all plugs *within* a sample.) Fish with scales must be scaled (and any adhering slime should be removed) prior to filleting. Scale a fish by laying it flat on a clean glass cutting board and scraping from the tail to the head using a stainless steel scaler or the blade-edge of a clean stainless steel knife.

10. Turn the fish sample so that the left side is facing up. Insert a new 8-mm biopsy punch into the fish through the tissue in the dorsal (upper) portion of the specimen between the dorsal fin and the lateral line, avoiding areas where the punch may contact the viscera (internal organs). Insert the punch with a slight twisting motion, cutting the skin and muscle tissue. Once the punch is inserted to its full depth, use a slight bending or tilting motion of the punch to break off the end of the sample.

11. Remove the biopsy punch, taking care to ensure that the sample remains in the punch.

12. Place a laboratory pipette bulb on the end of the biopsy punch and squeeze the bulb quickly, blowing the tissue sample into a tared clean 20-mL scintillation vial.

13. Repeat Steps 10 (sentences 3 and 4) through 13 to obtain a second plug of fillet tissue. The same biopsy punch used for the first plug extraction is used for the second plug extraction.

14. After transferring the second plug to the tared vial, weigh the tared vial containing the two plugs and determine the combined weight of the plugs by difference. Label the vial with the laboratory-extracted plug sample ID, the total weight of the two fillet plugs, and the date the sample was collected. Note that each plug sample consists of two plugs of fillet tissue.

Note: The two punch samples should yield at least 1.0 to 1.5 grams of fillet tissue for mercury analysis.

15. Transfer the vial to the freezer within 30 minutes. (The vial may be stored in a small cooler in the sample processing area on water ice or dry ice while the remainder of the five replicate plug samples are collected.)

16. Repeat steps 10 through 15 four times to collect a total of 5 replicate plug samples. Use the same biopsy punch to collect all 5 plug sample replicates, but discard it after completing collection of these 5 plug samples.

17. Filleting of the fish sample can proceed after all scales have been removed from the skin and a separate clean cutting board and fillet knife are prepared or available.

18. Put on new powder-free nitrile gloves. Place each fish on a clean glass cutting board in preparation for the filleting process. Note that filleting should be conducted under the supervision of an experienced fisheries biologist. Ideally, fish should be filleted while ice crystals are still present in the muscle tissue. Fish should be thawed only to the point where it becomes possible to make an incision into the flesh. Remove both fillets (lateral muscle tissue with skin attached) from the fish specimen using clean, high-quality stainless steel knives. Include the belly flap (ventral muscle and skin) with each fillet. Care must be taken to avoid contaminating fillet tissues with material released from inadvertent puncture of internal organs. In the event that an internal organ is punctured, rinse the fillet with deionized water immediately after filleting and make a note on the laboratory project log sheet that a puncture has occurred. Bones still present in the tissue after filleting should be carefully removed using the tip of the fillet knife or a clean pair of forceps.

19. Whole fillet samples (consisting of the entire right and left fillets) are weighed to the nearest gram (wet weight) and the weight is recorded on the bench sheet prior to homogenization. These samples should be homogenized partially frozen for ease of grinding.

20. Process each whole fillet sample using a size-appropriate homogenization apparatus (e.g., automatic grinder or high-speed blender). Entire fillets (with skin and belly flap) from both sides of the fish must be homogenized. Mix the tissues thoroughly until they are completely homogenized as evidenced by fillet tissue that consists of a uniform color and finely ground texture. Chunks of skin or tissue will hinder extraction and digestion and, therefore, are NOT acceptable. Grinding of tissue may be easier when tissues are partially frozen. Chilling the grinder briefly with a few small pieces or pellets of dry ice may also keep the tissue from sticking to the equipment. Pellets of dry ice also may be added to the tissue as it enters the grinder.

Note: The dry ice pellets used for homogenizing the fillet tissue are classified as food grade and meet the specifications for substances Generally Regarded As Safe as a direct food ingredient in the Food and Drug Administration regulation FDA 184.1240 (or 21CFR184.1240 in the Code of Federal Regulations).

21. Grind the entire fillet sample a second time, using the same grinding equipment. This second grinding should proceed more quickly. The grinding equipment does not need to be cleaned between the first and second grinding of the sample. The final homogenized fillet sample must consist of finely ground tissue of uniform color and texture. If there are obvious differences in color or texture, grind the entire sample a third time.

22. Measure the collective weight of the homogenized fillet tissue from each fish to the nearest gram (wet weight) after processing and record the total homogenate weight on a laboratory project log sheet. The collective weight of the homogenized tissue from each fish sample is transferred to spreadsheets for submission to the EPA Project Manager. At least 570 g of homogenized tissue will be needed to fill all of the containers in Table 1 below with their minimum acceptable masses. **If a sample does not yield at least 570 g of homogenized tissue, contact the EPA Project Manager via email immediately and await instructions.** As appropriate, place any remaining homogenized fillet tissue in the freezer while waiting for instructions, which are likely to involve preparing fewer archive aliquots.

23. After the final (second or third) grinding, clean the **grinding equipment and all other sample preparation equipment** using the procedures described in Step 29.

24. Once in every fish sample preparation batch (containing 2 whole fish samples), verify the continued absence of equipment contamination and uniformity of homogenization using the procedures described in Steps 32 to 36.

II.D. Aliquoting and Distribution Procedures

25. The sample preparation laboratory prepares the bulk homogenate tissue from one whole fish sample and uses it to fill the pre-cleaned sample containers specified for each type of aliquot listed in Table 1, following the procedures described in Step 26. **Except as noted in Table 1, all containers are provided by the fish sample preparation laboratory.** Documentation of their cleanliness provided by the vendor (i.e., certificates of analysis) must be retained by the fish sample preparation laboratory and provided to EPA on request. The target masses listed in Table 1 are designed to provide enough tissue for multiple analyses of each sample, including tissue for QC purposes, as needed. The fish sample preparation laboratory should not exceed those aliquot target masses when filling the containers. The order of the containers and target masses in Table 1 are important and are designed to ensure that adequate tissue is available for all analyses, as well as archiving.

Table 1. FPES Mercury Phase Fillet Sample Aliquot Requirements

Analysis	Target Mass	Container Type	Destination
Mercury, plug 1	1.0 - 1.5 g	20-mL glass scintillation vial	ALS Environmental, (Kelso, WA)
Mercury, plug 2	1.0 - 1.5 g	20-mL glass scintillation vial	ALS Environmental, (Kelso, WA)
Mercury, plug 3	1.0 - 1.5 g	20-mL glass scintillation vial	ALS Environmental, (Kelso, WA)
Mercury, plug 4	1.0 - 1.5 g	20-mL glass scintillation vial	ALS Environmental, (Kelso, WA)
Mercury, plug 5	1.0 - 1.5 g	20-mL glass scintillation vial	ALS Environmental, (Kelso, WA)
Mercury, fillet 1	5 - 10 g	50-mL HDPE straight-sided jar, or conical HDPE tube with snap top	ALS Environmental, (Kelso, WA)
Mercury, fillet 2	5 - 10 g	50-mL HDPE straight-sided jar, or conical HDPE tube with snap top	ALS Environmental, (Kelso, WA)
Mercury, fillet 3	5 - 10 g	50-mL HDPE straight-sided jar, or conical HDPE tube with snap top	ALS Environmental, (Kelso, WA)
Mercury, fillet 4	5 - 10 g	50-mL HDPE straight-sided jar, or conical HDPE tube with snap top	ALS Environmental, (Kelso, WA)
Mercury, fillet 5	5 - 10 g	50-mL HDPE straight-sided jar, or conical HDPE tube with snap top	ALS Environmental, (Kelso, WA)
Lipid, fish 1	10 - 15 g	Container provided by the analytical laboratory	ALS Environmental, (Kelso, WA)
Lipid, fish 2	30 - 35 g	Container provided by the analytical laboratory	ALS Environmental, (Kelso, WA)
Small Archive 1	50 - 60 g	125-mL straight-sided amber or clear glass jar	CSRA Sample Repository, (Baltimore, MD)
Small Archive 2	50 - 60 g	125-mL straight-sided amber or clear glass jar	CSRA Sample Repository, (Baltimore, MD)
Bulk Archive 1	200 - 220 g	500-mL straight-sided amber or clear glass jar with foil-lined lid	CSRA Sample Repository, (Baltimore, MD)
Bulk Archive 2	200 - 220 g	500-mL straight-sided amber or clear glass jar with foil-lined lid	CSRA Sample Repository, (Baltimore, MD)
Total (to the nearest gram)*	570 - 667.5 g		

* In the event that insufficient fish tissue mass exists to prepare the required number of aliquots, contact the EPA Project Manager for instructions, per Step 22.

26. Prepare the plug samples and homogenized sample aliquots for **mercury**. Weigh an appropriate clean sample container (Table 1) to the nearest 0.5 g and record the weight. Transfer sufficient fillet plug tissue or homogenized fillet aliquots to the container to achieve the target mass for that container in Table 1, weigh the container again, record the weight, and determine the weight of the aliquot to the nearest 0.5 g by difference.

Note: The archive sample jars are not filled until after sufficient volume for lipids determination has been collected, as described in Step 28. For the sample used for homogeneity testing, the archive jars are not filled until triple the lipid mass is collected (see Step 35).

When filling jars, leave sufficient space at the top of each jar before sealing with the designated lid to allow for expansion of the tissue as it freezes. *In no case should jars be filled beyond 80% capacity, as this may result in breakage on freezing.* Wipe off the outside of the jars to remove any tissue residue or moisture. Fill out a label for each container using a waterproof marker. Include the following information (at a minimum) on each label:

- sample identification number,
- tissue sample type (e.g., fillet plug or homogenized fillet)
- analysis type (e.g., mercury),
- plug or aliquot weight (to the nearest 0.5 gram),
- preparation batch ID (assigned by EPA as a numerical sequence from 16 to 45), and
- preparation date (e.g., mm/dd/yyyy)

Affix the label to the container with clear wide tape. Place each container inside one heavy-weight food-grade self-sealing plastic freezer bag to avoid sample loss due to breakage. Freeze the tissue samples at -20 °C, and maintain samples in the freezer until directed by the EPA Project Manager to ship them to the analytical laboratories. (The EPA Project Manager will not issue these instructions until equipment rinsate and homogeneity tests described in Steps 29 to 36 have been completed, reported, evaluated, and determined to be acceptable.)

27. After filling all of the containers with the tissue samples for mercury, remove 10 to 15 g of homogenized fillet tissue from fish 1 in the batch (for single lipid analysis) and 30 to 35 g of homogenized fillet tissue from fish 2 (for triplicate lipid analysis) to be used to determine the lipid content of each sample fillet. Place these aliquots in clean glass or plastic containers of suitable size (provided by ALS Environmental, Kelso, WA) and label each of them with the sample ID number. Store the lipid aliquots in the freezer at -20°C until they are ready to be shipped to the designated analytical laboratory to perform the lipid determinations in Steps 31, 35, and 36.

28. The archive sample jars are not filled until after sufficient volume for determining lipids has been collected. Once the aliquots for mercury and lipids have been collected, the remaining tissue mass is used to create the four archive samples. Begin by transferring 50 - 60 g of tissue to the first small archive sample container. Continue by transferring a 50 - 60 g aliquot to the remaining small archive container. Ideally, sufficient homogenized fillet tissue mass will remain to produce two bulk archive containers. Therefore, transfer 200 - 220 g of tissue to the first bulk archive sample container. Continue by transferring 200 - 220 g of tissue to the second bulk archive container. However, if less than 220 g of tissue is available, transfer all of the remaining homogenized tissue to the bulk archive container. Seal and label the containers as described in Step 26 for the other aliquots.

    Note: Step 22 requires that the laboratory contact the EPA Project Manager whenever a sample does not yield at least 570 g of tissue. The EPA Project Manager will provide direction to the laboratory regarding samples yielding less than 570 g of tissue that must be followed at this point in the procedure.

    Any tissue that remains after filling the second bulk archive jar may be discarded.

II.E. Equipment Cleaning between Composite Samples

29. All of the homogenization equipment must be thoroughly cleaned between each individual fish sample. Once both of the fillets from the individual sample have been homogenized, disassemble the homogenization equipment (i.e., blender, grinder, or other device) and thoroughly **clean all surfaces and parts** that contact the sample. Similarly, **clean all knives, cutting boards, and other utensils used**. At a minimum:

    - Wash with a detergent solution (phosphate- and scent-free) and warm tap water
    - Rinse three times with warm tap water
    - Rinse three times with deionized (DI) water
    - Rinse with acetone
    - Rinse three times with DI water
    - Rinse with (not soak in) 5% nitric acid
    - Rinse three times with DI water
    - Allow the components to air dry

30. Reassemble the homogenization equipment and proceed with homogenization of the next fish sample in the batch (e.g., begin with Step 6 above).

II.F. Lipid Determination for Every Homogenized Fillet Sample

The procedures for determining the lipid content of every homogenized fillet sample from fish 1 in a fish sample preparation batch are described in Step 31 below. (Additional lipid determinations are required for fish 2 in every fish sample preparation batch, as described in Steps 35 and 36.)

31. For fish 1 in each fish sample preparation batch, use the 10 to 15 g aliquot of homogenized tissue collected in Step 27 to determine the lipid content of the sample. ALS Environmental (Kelso, WA) will extract the aliquot using SW-846 Method 9071B to determine the lipid content of that aliquot, which is recorded in units of percent (i.e., grams of lipid per gram of tissue x 100).

II.G. Quality Control (QC) Procedures

The project-specific QC procedures include preparation and testing of equipment rinsate samples and homogeneity testing, using lipids as a surrogate.

During the sample preparation efforts, the fish sample preparation laboratory will prepare one set of mercury rinsate samples and one homogenized fillet tissue aliquot for triplicate lipid determinations from one of every two fish samples represented in each fish sample preparation batch, as described in Steps 32 to 35 below. The batch-specific rinsate and homogeneity results will be reviewed by Tetra Tech and CSRA. The fish sample preparation laboratory may continue to process up to 5 additional batches during the QC sample analysis and review process. However, the fish sample preparation laboratory may **not** continue beyond that series of 6 batches of samples until receiving notification from the EPA Project Manager that review of the initial batch rinsate and homogeneity test results is complete and the results were deemed satisfactory.

Continued sample processing is dependent on both the quality of the fish sample preparation laboratory's efforts and on the timeliness of their delivery of QC results.

Rinsate and Blank Sample Production

32. Once per batch (of 2 fish) during the fish sample preparation operations, prepare a set of rinsate samples prior to reassembling the homogenization equipment (Step 30), as follows:

    - Prepare a **DI water rinsate** using 250 mL of DI water. Collect the DI water rinsate in a clean glass or HDPE container.
    - Place a second aliquot of DI water in a separate similar clean container for use as a blank.
    - Acidify these two samples to pH < 2 with nitric acid. This set of rinsate and blank samples will be analyzed for mercury as described in Step 34.

33. Label each container as either "rinsate -- DI water" or "blank -- DI water," and include the date it was prepared (mm/dd/yyyy), the analysis type (Hg), and the preparation batch identifier. Store the rinsates and blanks in a refrigerator at a temperature <6 °C.

Rinsate Analyses

34. During the fish sample preparation operations, a laboratory under contract to Tetra Tech (ALS Environmental) will analyze one set of DI water rinsate and blank samples per batch for mercury using EPA Method 245.1, a cold-vapor atomic absorption procedure.

Corrective Actions for Rinsates

The rinsate results will be evaluated based on the mass of mercury detected, and assuming that all of the apparent contamination could be transferred to a nominal 50-g mass of homogenized tissue. Results for mercury above the anticipated reporting limits for this analyte in homogenized fillet tissue samples may be cause for corrective actions by the fish sample preparation laboratory. These corrective actions may include revisions to the fish sample preparation laboratory's equipment cleaning procedures, followed by a successful demonstration of the revised cleaning procedures through preparation and analysis of additional rinsate samples.

Lipid Determination to Confirm Homogeneity

35. For fish 2 in each fish sample preparation batch, a laboratory under contract to Tetra Tech will use the 30 - 35 g aliquot of homogenized fillet tissue to conduct triplicate analyses of the lipid content of homogenized fillet tissue samples to confirm that they are homogeneous.

    Remove 30 to 35 g of fillet tissue before filling the archive sample containers. Place this aliquot in a glass or plastic container of suitable size and label it with the sample ID number. Transfer the lipid aliquot to ALS Environmental, Kelso, WA for triplicate lipid determination. This laboratory will use 5 to 10 g aliquots of fillet tissue for each of the 3 lipid analyses.

36. From the lipid results, calculate the mean lipid content (in percent), the standard deviation (SD), and the relative standard deviation (RSD) using the formulae below, or the corresponding functions in Excel.

$$\text{mean \%lipids} = \frac{\sum_{i=1}^{3}(\%\,\text{lipids})_i}{3}$$

$$SD = \sqrt{\frac{\sum_{i=1}^{3}(\%\,\text{lipids}_i - \text{mean lipids})^2}{2}}$$

$$RSD = \frac{SD}{\text{mean}}$$

If the RSD of the triplicate results is less than or equal to 15%, then the homogenization effort is judged to be sufficient for all samples in that preparation batch. For this sample analyzed in triplicate, the mean lipid content will be the value reported for that sample, following the requirements described in Step 31.

Corrective Actions for Homogeneity

If the RSD is greater than 15%, then corrective action is required for all samples in that preparation batch. Corrective actions will be determined by EPA in direct consultation with the laboratory and Tetra Tech, but the default corrective action consists of regrinding all of the aliquots from each whole fish sample in the affected batch until the RSD criterion is met.

This may entail retrieving all sample aliquots (see Table 1) from the freezer, allowing them to partially thaw, and homogenizing them again, beginning at Step 20. In these instances, all of the equipment cleaning procedures will be repeated between each whole fish sample, new lipid results will be determined for each fish sample, and a new homogenization QC determination (triplicate lipids on one fish sample per batch) will be performed. New sample containers are required for any rehomogenized samples.

II.H. Reporting Requirements

37. The sample preparation laboratory will prepare a weekly progress report to document the status of fish preparation activities and forward the report electronically to EPA. The format of the weekly progress report will be as an Excel spreadsheet using the 2015 Great Lakes Human Health Fish Fillet Tissue Sample fish sample preparation as a guide for organization of the spreadsheet. For each homogenized sample processed or plug sample collected during that period, include at least the following information in the report:

- sample identification number,
- common name for the fish species (provided to the laboratory in the instructions from EPA),
- field-determined length and lab-determined weight of each fish sample,
- total whole fillet (unhomogenized) weight (to the nearest gram),
- homogenized fillet sample (i.e., homogenate) weight (to the nearest gram),
- total laboratory-extracted plug sample weight (to the nearest 0.1 gram),
- analysis type (e.g., mercury, lipid, and archive samples),
- aliquot weight (to the nearest 0.5 gram),
- preparation batch ID,
- preparation date (e.g., mm/dd/yyyy),

- QC sample identifiers associated with the batch of homogenized fillet samples, and
- lipid results for each fish sample

The weekly report will be due by COB Monday (or one day later in the case of holidays) and it will document sample preparation progress for the previous week.

In addition, the laboratory must report the results of the rinsate analyses for mercury and the triplicate lipid results associated with the sample batch. Those results **must** be reported to EPA as soon after the analyses as practical to facilitate timely review and to minimize delays in receiving approval to process future batches.

Note: As specified in the QC section of this QAPP, the fish sample preparation laboratory may **not** continue beyond the series of 6 fish sample preparation batches until receiving notification from EPA that review of the initial (in the series of 6 batches) batch rinsate and homogeneity test results is complete and the results were deemed satisfactory.

II.I. Shipping Samples

38. **No samples may be shipped until the EPA Project Manager has reviewed the sample homogeneity testing and rinsate results.** The EPA Project Manager will notify the fish sample preparation laboratory by email when specific samples may be shipped, and to whom.

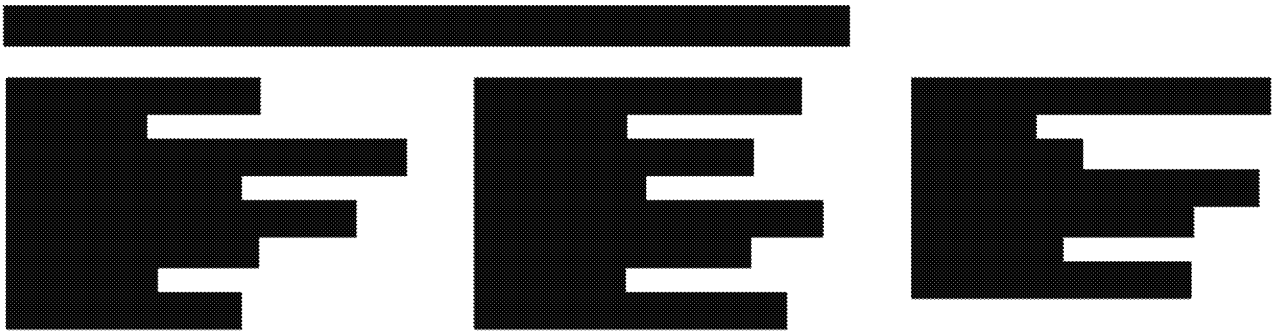
    Samples are shipped in batches (one batch per cooler) to the designated analytical laboratory. Each mercury analysis batch contains 10 lab-extracted plug samples and 10 homogenized fillet tissue samples (5 replicates of each type of sample per fish) from two whole fish samples.

    When shipping batches of pre-frozen fillet tissue aliquots, keep the individual containers bagged in the food-grade plastic freezer bags. Place these bags in a cooler with adequate space for the tissue containers, packing materials, and dry ice. Shipments of plug samples for mercury analysis may use other forms of packing materials (e.g., bubble-wrap bags, foam blocks with pre-drilled holes) appropriate for the scintillation vials.

    Secure each of the tissue containers with packing materials (e.g., bubble wrap or foam) before adding the dry ice. Place a layer of bubble wrap and a plastic cooler liner on top of the containers before adding the dry ice, as this can prevent cracking the lids.

    The amount of dry ice required for shipping will depend on the number of homogenized fillet tissue samples and lab-extracted plug samples in the cooler and the time of year. It should be an adequate supply to keep the tissue samples frozen for 48 hours (i.e., a minimum of 30 pounds of dry ice per cooler for up to 10 pounds of fillet tissue samples).

    Record the samples contained in the cooler on a shipping form provided by CSRA and place the form in a plastic bag taped to the inside lid of the cooler. Secure the outside of the cooler with sealing tape, address it to the sample recipient identified by the EPA Project Manager, and attach a dry ice (dangerous goods) label. Ship the cooler via an overnight express carrier on a date that will allow delivery of the cooler to the analytical laboratory on a normal business day (e.g., **no Saturday deliveries and no deliveries on U.S. Federal holidays**). Provide the air bill number for each shipment to CSRA and the EPA Project Manager via email on the day that the shipment occurs. **CSRA will provide the fish sample preparation laboratory with a third-party FedEx account to which each shipment will be billed.**



ATTACHMENT 1
ANALYSES OF RINSATES AND BLANKS FOR MERCURY

This attachment describes the analyses of rinsate samples and blanks generated during the fish sample preparation process. The results of those analyses are important in demonstrating that the sample preparation laboratory's equipment cleaning procedures are effective at preventing cross-contamination between fish tissue samples.

A. EQUIPMENT AND MATERIALS:

- Mercury analyzer suitable for aqueous samples using cold-vapor atomic absorption (CVAA) instruments compatible with EPA Method 245.1 (must be capable of achieving an MDL of approximately 1 µg/L).
- Assorted glassware, syringes, etc.

B. RINSATE AND BLANK ANALYSES

Each set of rinsate samples will include:

- One deionized water (DI) rinsate sample and one DI water blanks sample for analysis of mercury.

During sample preparation efforts, the laboratory will prepare mercury rinsates at a frequency of one set for each batch of 2 fish samples prepared. Thirty sets of rinsates are anticipated.

The analytical laboratory (ALS Environmental) will digest and analyze the mercury rinsates and blanks by CVAA. For each analysis, the laboratory will determine the mass of mercury in the total volume of each rinsate or blank sample, rather than the concentration of mercury.

ALS Environmental will either perform a method detection limit (MDL) study for mercury in aqueous samples, or use existing aqueous MDL data for the CVAA instrument employed. The laboratory must be able to achieve an MDL of approximately 1 µg/L. Mercury results will be reported down to the mass equivalent to the mass at the method detection limit (MDL) for aqueous samples.

C. QUALITY CONTROL

The quality control (QC) procedures required for the rinsate analyses for mercury include:

- MDL studies, as described above
- Instrument calibration (see Method 245.1 for procedures and acceptance criteria)
- Instrument blanks
- Calibration verification (once per analysis batch consisting of one set of rinsate samples or a total of 30 analysis batches)
- Laboratory control sample (LCS) once per analysis batch (consisting of one set of rinsate samples)

The MDL results will be reviewed by CSRA and EPA as soon as they become available for each fish sample preparation batch, and the laboratory will not be authorized to prepare fish tissue samples beyond a series of 6 fish sample preparation batches until that review is complete and the results are acceptable for the initial batch in each series of 6 fish sample preparation batches.

The matrix for the mercury rinsates is reagent (deionized) water, which should not adversely affect method performance. Therefore, matrix spike samples are not required for mercury.

The instrument blanks for mercury take the place of a traditional method blank that would be extracted along with environmental samples.

D. DELIVERABLES

Summary data from the rinsate analyses are to be delivered to EPA in an Excel file. That file must contain the following information, at a minimum:

- Batch ID - assigned by EPA (numerical sequence from 16 to 45)
- Sample ID - as described in the instructions for preparing the rinsates
- Lab sample ID - unique internal identifier used by the laboratory, if any
- Prep date - Date (MM/DD/YYYY) on which the rinsate or solvent blank was prepared
- Analysis type - "Mercury"
- Analysis date - Date (MM/DD/YYYY) on which the rinsate or solvent blank was analyzed
- Analyte name – Mercury (total)
- Mass of mercury found - in micrograms
- Lab qualifiers - as needed to describe any analytical concerns. A complete list of the qualifiers and their meanings must be included with each data submission (e.g., in a separate tab on the Excel file).
- Reporting limit (i.e., the MDL for this study) for mercury - in the same mass units used for the mercury results
- Instrument calibration data - Submit as a separate tab in the Excel file. Must include results for the initial calibrations for mercury, as well as any relevant calibration verifications associated with the mercury analyses. Include calibration equations (e.g., regressions) and metrics (e.g., correlation coefficient or calibration factor).

Provide Excel files for the mercury analysis results to the Tetra Tech Project Leader. Raw data supporting mercury analysis (e.g., instrument printouts) must be retained by the laboratory and made available to EPA when requested. If requested, raw data may be submitted in hard copy, or as a PDF file.

Appendix C

Fish Plug Evaluation Study Mercury Phase Fish Sample Preparation Laboratory Bench Sheet

FPES Fish Sample Preparation Laboratory Bench Sheet

Page ___of___

Site ID:	Filleter:	Fish _____
EPA Batch ID:	Tissue Processor:	
Prep Date (MMDDYYYY):		

Sample Type	Sample ID	Species	Fish Length (mm)	Fish Mass (g)	Fillet Mass (g)	Fillet Tissue Recovery (%)	Total Homogenate Mass (g)	Homogenate Tissue Recovery (%)
Fillet								
Notes:								

Sample Jar	Hg Plug 1 (2 plugs)	Hg Plug 2 (2 plugs)	Hg Plug 3 (2 plugs)	Hg Plug 4 (2 plugs)	Hg Plug 5 (2 plugs)	Hg Fillet 1	Hg Fillet 2	Hg Fillet 3	Hg Fillet 4	Hg Fillet 5
Sample ID										
Target Sample Mass (g)	1.0 - 1.5 g	1.0 - 1.5 g	1.0 - 1.5 g	1.0 - 1.5 g	1.0 - 1.5 g	5 - 10 g	5 - 10 g	5 - 10 g	5 - 10 g	5 - 10 g
Sample Mass (g)										

Sample Jar	Lipids (Fish 1 from each batch)	Triplicate Lipids (Fish 2 from each batch)	Small Archive 1	Small Archive 2	Bulk Archive 1	Bulk Archive 2
Sample ID						
Target Sample Mass (g)	10 - 15 g	30 - 35 g	50 - 60 g	50 - 60 g	200 - 220 g	200 - 220 g
Sample Mass (g)						

Rinsate/Blank Samples (1 Fish Per Batch)	
Rinsate Sample	Blank Sample
rinsate -- DI water	blank -- DI water
Prepare using 250 mL water	Prepare using 250 mL water
Yes / No	Yes / No

Tetra Tech, Inc.
Ecological Testing Facility

Data Checked and Approved _____

2018

Appendix D

Fish Plug Evaluation Study Selenium Phase Fillet Tissue Preparation, Homogenization, and Distribution Procedures

Appendix D

Fish Plug Evaluation Study Selenium Phase
Fillet Tissue Preparation, Homogenization, and Distribution Procedures

I. PURPOSE

This document describes the procedures that the fish sample preparation laboratory follows when preparing fish fillet tissue samples for the selenium phase of EPA's Fish Plug Evaluation Study (FPES) under contract to EPA. Adherence to these procedures ensures that fish tissue preparation activities at the Tetra Tech laboratory in Owings Mills, MD are performed consistently across all study samples and in a manner consistent with previous EPA fish tissue studies. The effort is divided into two primary components:

- Fish fillet tissue processing and distribution procedures, including quality control steps, for the 6 fish sample preparation batches.
- Analyses of rinsate and blank samples for selenium (6 pairs corresponding to each of the fish sample preparation batches).

Each of these components is described in detail below.

II. FISH FILLET TISSUE PROCESSING AND DISTRIBUTION PROCEDURES

The procedures for processing and distributing FPES fillet tissue samples are described below. The process description is organized into the following components, including the quality control (QC) procedures:

A. Sample Receipt and Storage
B. Sample Handling
C. Filleting and Homogenization Procedures, Including Removal of Plug Samples for Selenium and Percent Solids Analyses
D. Aliquoting and Distribution Procedures
E. Equipment Cleaning between Fish Samples
F. Lipid Determination on Every Homogenized Fillet Sample
G. Quality Control (QC) Procedures
H. Reporting Requirements
I. Shipping Samples

The individual steps in the overall process are presented as a series of numbered steps across the nine components listed above.

Fillet Tissue Processing Definitions

- **Whole Fish Sample:** A whole fish sample for the Fish Plug Evaluation Study consists of the single fish sample collected for each target species at the 6 designated sampling locations (3 Great Lakes, including Lake Erie, Lake Michigan, and Lake Ontario, and 3 mid-Atlantic rivers, including the Anacostia River, Potomac River, and St. Lawrence River). For the selenium phase of the study, 5 individual whole fish samples are collected from each sampling location as follows: Lake Erie, walleye; Lake Michigan, lake trout; Lake Ontario, Chinook salmon; Anacostia River, blue catfish; Potomac River, largemouth bass; and St. Lawrence River,

smallmouth bass. Field crews collect a total of 30 whole fish samples during the selenium phase of the study and transport or ship them to the fish sample preparation laboratory (Tetra Tech laboratory, Owings Mills, MD).

- **Fillet plug samples:** A fillet plug sample for selenium analysis consists of 2 plugs of fillet tissue that are removed from a whole fish sample using an 8-mm biopsy punch. A fillet plug sample for percent solids analysis consists of 1 plug of fillet tissue that is removed from a whole fish sample using an 8-mm biopsy punch. For the selenium phase of the study, 4 selenium and 4 percent solids fillet plug samples are removed from a whole fish sample in the field and another 4 selenium and 4 percent solids fillet plug samples are removed from that whole fish sample in the fish sample preparation laboratory. Each selenium plug sample should contain 1.0 to 1.5 grams of fillet tissue and each percent solids plug sample should contain 0.50 to 0.75 grams of fillet tissue. The selenium phase of the Fish Plug Evaluation Study will generate 120 pairs of field-extracted selenium and percent solids fillet plug samples and 120 pairs of lab-extracted selenium and percent solids fillet plug samples for selenium analysis and percent solids analysis, respectively.

- **Fish sample preparation batch:** Each fish sample preparation batch consists of 5 individual whole fish samples. The 30 whole fish samples are assigned to 6 fish sample preparation batches (based on chronological order that fish samples are collected, beginning with the oldest samples first). The selenium phase fish processing instructions are the same for each of the 6 fish sample preparation batches. Processing the 5 whole fish samples in the fish sample preparation batch involves removal of 4 replicate double-plug and 4 replicate single-plug fillet samples and preparation of 4 replicate homogenized fillet tissue samples (with sufficient tissue for both the selenium and percent solids analyses) from each of the fish in the batch. Each fish sample preparation batch produces a total of 20 double-plug fillet samples and 20 single-plug fillet samples for selenium and percent solids analyses, respectively, along with 20 homogenized fillet tissue samples for both selenium and percent solids analyses.

- **Selenium analysis batch:** A selenium analysis batch consists of 20 fillet tissue samples. There are 2 selenium analysis batches generated during processing of a fish sample preparation batch: 20 double-plug fillet tissue samples extracted in the lab (4 replicate samples per fish) and 20 homogenized fillet tissue samples (4 replicates per fish) from the 5 whole fish samples in a fish sample preparation batch.

- **Percent solids analysis batch**: For every selenium analysis batch, there is a corresponding batch of fillet samples prepared for percent solids analysis. This includes single-plug fillet samples that correspond to each of the field and lab plugs collected for selenium analysis. It also includes extra homogenized fillet tissue mass in each selenium analysis aliquot for percent solids analysis. The corresponding pairs of selenium and percent solids fillet samples for each of the two 20-sample batches generated from a selenium phase fish sample preparation batch are shipped to the designated analytical lab (Brooks Applied Labs in Bothell, WA) in a single cooler.

II.A Sample Receipt and Storage

Tetra Tech field crews are collecting the fish samples for the selenium phase of the Fish Plug Evaluation Study during June and July 2018. Thirty (30) fish are being collected for the selenium phase of this study, consisting of 5 individual fish samples for each of six species (three Great Lakes species and three mid-Atlantic river species).

Samples are hand-delivered to the sample preparation laboratory for storage and processing. The sample preparation laboratory must have sufficient freezer space to store **up to 30 unprocessed fish samples** at a temperature of less than or equal to -20 °C from the time of receipt until completion of sample processing and sufficient freezer space to store **fillet plug samples and homogenized fillet tissue aliquots from up to 30 processed samples** (e.g., up to 630 jars) prior to distribution.

1. Although samples are delivered frozen, on dry ice, they must be inspected promptly on receipt. As samples are received, the sample custodian must:

    - Check that each shipping container has arrived undamaged and verify that samples are still frozen and in good condition.
    - Check the temperature of one of the samples in the cooler using a thermometer that reads to at least -20 °C, or an infra-red (IR) temperature "gun," and record the reading.
    - Verify that all associated paperwork is complete, legible, and accurate.
    - Compare the information on the label on each individual fish sample and on each field-extracted fillet plug sample to the sample tracking form to verify that each specimen was included in the shipment and is properly wrapped and labeled.
    - Notify the EPA Project Manager of the fact that samples were received on the day of delivery and report any discrepancies in the paperwork identified above.
    - Check that the samples were collected from the designated Great Lakes or river sites and notify the EPA Project Manager of any changes in sampling locations.
    - Transfer the samples to the freezer for long-term storage.

2. Notify the EPA Project Manager immediately about any problems encountered upon receipt of samples. Problems involving sample integrity, conformity, or inconsistencies for fish samples should be reported to the EPA Project Manager in writing (e.g., by email) within one business day following sample receipt and inspection.

    Following fillet sample processing, the fish sample preparation laboratory must store fillet plug samples and homogenized fillet tissue sample aliquots frozen to less than or equal to -20 °C until they are distributed to the laboratory designated for selenium analysis of FPES fillet samples (Brooks Applied Labs in Bothell, WA).

II.B Sample Handling

The whole fish samples collected for the FPES must remain frozen at less than or equal to -20 °C until the fish sample preparation laboratory receives direction from the EPA Project Manager to begin processing the samples. Samples to be processed must be retrieved from the freezer, with their associated paperwork, and allowed to partially thaw before they can be processed.

3. The fish sample processing instructions are the same for all 6 fish sample preparation batches. Each fish sample preparation batch consists of 5 whole fish samples. Prior to beginning fish sample processing, Tetra Tech prepares an Excel spreadsheet assigning whole fish samples to fish sample preparation batches and submits the spreadsheet for approval by the EPA Project Manager. Processing for a fish sample preparation batch involves the following for each fish in the batch:

    - Removal of 4 replicate selenium fillet plug samples (2 plugs per plug sample) and 4 replicate percent solids fillet plug samples (1 plug per sample)
    - Preparation of 4 replicate homogenized fillet tissue samples.

Note: Processing a fish sample preparation batch produces 20 double-plug fillet samples and 20 single-plug fillet samples, which constitutes one selenium analysis batch with paired percent solids plug samples. It also produces a second selenium analysis batch of 20 homogenized fillet samples with sufficient additional tissue to do the paired percent solids analysis.

4. When retrieving samples from the freezer, the sample custodian must:

   - Verify that all associated paperwork stored with the samples is complete, legible, and accurate.
   - Compare the information on the label on each individual fish sample to the fish sample preparation batch spreadsheet and notify the EPA Project Manager of any discrepancies between the sample labels and this Excel file. Problems involving sample paperwork, sample integrity, or custody information inconsistencies for all fish samples should be reported to the EPA Project Manager in writing (e.g., by email) within one business day following sample retrieval and inspection. **Do not proceed with sample processing until discrepancies are resolved.**

II.C. Filleting and Homogenization Procedures, Including Removal of Plug Samples for Selenium Analysis and for Percent Solids Analysis

Consistent with the mercury phase, FPES selenium and percent solids analyses are performed on three types of fillet tissue samples: field-extracted fillet plug samples, laboratory-extracted fillet plug samples, and aliquots of homogenized fillet tissue samples. Sample processing includes collection of 4 pairs of plug samples (one sample consisting of 2 plugs for selenium analysis and the other sample consisting of one plug for percent solids analysis) from each whole fish sample in the fish sample preparation batch, using the procedures described in Steps 10 - 17. Steps 5 - 9 below must be completed before beginning collection of fillet plug samples.

5. Prior to preparing any fillet samples, thoroughly clean utensils and cutting boards using the following series of procedures:

   - Wash with a detergent solution (phosphate- and scent-free) and warm tap water
   - Rinse three times with warm tap water
   - Rinse three times with deionized (DI) water
   - Rinse with acetone
   - Rinse three times with DI water
   - Rinse with (not soak in) 5% nitric acid
   - Rinse three times with DI water

   To control contamination, separate sets of utensils and cutting boards must be used for scaling fish and for filleting fish.

   Note: A new biopsy punch is used for collecting the series of 8 plug samples from each whole fish sample (4 for selenium analysis and 4 for percent solids analysis), then discarded. Biopsy punches are **not** subjected to the cleaning procedures above.

6. Put on powder-free nitrile gloves before unpacking an individual fish sample for plug sample collection and for filleting and tissue homogenization. After unwrapping, inspect each fish carefully to verify that it has not been damaged during collection or shipment. If damage (e.g., tearing the skin or puncturing the gut) is observed, document it in the laboratory project log sheet and notify the EPA Project Manager before proceeding further.

7. Weigh each fish to the nearest gram (wet weight) prior to any sample processing. Enter weight information for each individual fish into a laboratory project log sheet. Individual fish sample weights eventually will be transferred to spreadsheets for submission to the EPA Manager.

8. Rinse each fish with deionized water as a precautionary measure to treat for possible contamination from sample handling in the field. Use HDPE wash bottles for rinsing fish and for cleaning homogenization equipment and utensils.

9. Before beginning the scaling process for each fish, put on new powder-free nitrile gloves. (Gloves must be changed *between* individual fish samples, but the same gloves may be used for all plugs *within* a sample.) Fish with scales must be scaled (and any adhering slime should be removed) prior to filleting. Scale a fish by laying it flat on a clean glass cutting board and scraping from the tail to the head using a stainless steel scaler or the blade-edge of a clean stainless steel knife.

10. Turn the fish sample so that the left side is facing up. Insert a new 8-mm biopsy punch into the fish through the tissue in the dorsal (upper) portion of the specimen between the dorsal fin and the lateral line, avoiding areas where the punch may contact the viscera (internal organs). Insert the punch with a slight twisting motion, cutting the skin and muscle tissue. Once the punch is inserted to its full depth, use a slight bending or tilting motion of the punch to break off the end of the sample.

11. Remove the biopsy punch, taking care to ensure that the sample remains in the punch.

12. Place a laboratory pipette bulb on the end of the biopsy punch and squeeze the bulb quickly, blowing the tissue sample into a tared clean 20-mL scintillation vial.

13. Repeat Steps 10 (sentences 3 and 4) through 13 to obtain a second plug of fillet tissue. The same biopsy punch used for the first plug extraction is used for the second plug extraction.

14. After transferring the second plug to the tared vial, weigh the tared vial containing the two plugs for selenium analysis and determine the combined weight of the plugs by difference. Label the vial with the laboratory-extracted plug sample ID, the total weight of the two fillet plugs, and the date the sample was collected. Note that each selenium plug sample consists of two plugs of fillet tissue.

Note: The two punch samples should yield at least 1.0 to 1.5 grams of fillet tissue for selenium analysis.

15. Repeat steps 10 (sentences 3 and 4) through 12 to obtain a single-plug fillet sample for percent solids analysis, then repeat Step 14 after adapting the weighing and labeling instructions for only one plug in the vial.

Note: The one punch sample should yield at least 0.50 to 0.75 grams of fillet tissue for percent solids analysis.

16. Transfer the vials to the freezer within 30 minutes. (Each vial may be stored in a small cooler in the sample processing area on water ice or dry ice while the remainder of the four replicate plug samples are collected.)

17. Repeat steps 10 through 16 three times to collect a total of 4 replicate plug samples for selenium analysis and 4 replicate plug samples for percent solids analysis. Use the same biopsy punch to collect all 8 plug sample replicates, but discard it after completing collection of these 8 plug samples.

18. Filleting of the fish sample can proceed after all scales have been removed from the skin and a separate clean cutting board and fillet knife are prepared or available.

19. Put on new powder-free nitrile gloves. Place each fish on a clean glass cutting board in preparation for the filleting process. Note that filleting should be conducted under the supervision of an experienced fisheries biologist. Ideally, fish should be filleted while ice crystals are still present in the muscle tissue. Fish should be thawed only to the point where it becomes possible to make an incision into the flesh. Remove both fillets (lateral muscle tissue with skin attached) from the fish specimen using clean, high-quality stainless steel knives. Include the belly flap (ventral muscle and skin) with each fillet. Care must be taken to avoid contaminating fillet tissues with material released from inadvertent puncture of internal organs. In the event that an internal organ is punctured, rinse the fillet with deionized water immediately after filleting and make a note on the laboratory project log sheet that a puncture has occurred. Bones still present in the tissue after filleting should be carefully removed using the tip of the fillet knife or a clean pair of forceps.

20. Whole fillet samples (consisting of the entire right and left fillets) are weighed to the nearest gram (wet weight) and the weight is recorded on the bench sheet prior to homogenization. These samples should be homogenized partially frozen for ease of grinding.

21. Process each whole fillet sample using a size-appropriate homogenization apparatus (e.g., automatic grinder or high-speed blender). Entire fillets (with skin and belly flap) from both sides of the fish must be homogenized. Mix the tissues thoroughly until they are completely homogenized as evidenced by fillet tissue that consists of a uniform color and finely ground texture. Chunks of skin or tissue will hinder extraction and digestion and, therefore, are NOT acceptable. Grinding of tissue may be easier when tissues are partially frozen. Chilling the grinder briefly with a few small pieces or pellets of dry ice may also keep the tissue from sticking to the equipment. Pellets of dry ice also may be added to the tissue as it enters the grinder.

Note: The dry ice pellets used for homogenizing the fillet tissue are classified as food grade and meet the specifications for substances Generally Regarded As Safe as a direct food ingredient in the Food and Drug Administration regulation FDA 184.1240 (or 21CFR184.1240 in the Code of Federal Regulations).

22. Grind the entire fillet sample a second time, using the same grinding equipment. This second grinding should proceed more quickly. The grinding equipment does not need to be cleaned between the first and second grinding of the sample. The final homogenized fillet sample must consist of finely ground tissue of uniform color and texture. If there are obvious differences in color or texture, grind the entire sample a third time.

23. Measure the collective weight of the homogenized fillet tissue from each fish to the nearest gram (wet weight) after processing and record the total homogenate weight on a laboratory project log sheet. The collective weight of the homogenized tissue from each fish sample is transferred to spreadsheets for submission to the EPA Project Manager. At least 656 g of homogenized tissue will be needed to fill all of the containers in Table 1 below with their minimum acceptable masses. **If a sample does not yield at least 656 g of homogenized tissue, contact the EPA Project Manager via email immediately and await instructions.** As appropriate, place any remaining homogenized fillet tissue in the freezer while waiting for instructions, which are likely to involve preparing fewer archive aliquots.

24. After the final (second or third) grinding, clean the **grinding equipment and all other sample preparation equipment** using the procedures described in Step 30.

25. Once in every fish sample preparation batch (containing 5 whole fish samples), verify the continued absence of equipment contamination and uniformity of homogenization using the procedures described in Steps 33 to 37.

II.D. Aliquoting and Distribution Procedures

26. The sample preparation laboratory prepares the bulk homogenate tissue from one whole fish sample and uses it to fill the pre-cleaned sample containers specified for each type of aliquot listed in Table 1, following the procedures described in Step 27. **Except as noted in Table 1, all containers are provided by the fish sample preparation laboratory.** Documentation of their cleanliness provided by the vendor (i.e., certificates of analysis) must be retained by the fish sample preparation laboratory and provided to EPA on request. The target masses listed in Table 1 are designed to provide enough tissue for multiple analyses of each sample, including tissue for QC purposes, as needed. The fish sample preparation laboratory should not exceed those aliquot target masses when filling the containers. The order of the containers and target masses in Table 1 are important and are designed to ensure that adequate tissue is available for all analyses, as well as archiving.

Table 1. FPES Selenium Phase Fillet Sample Aliquot Requirements

Analysis	Target Mass	Container Type	Destination
Selenium, plug 1	1.0 - 1.5 g	20-mL glass scintillation vial	Brooks Applied Labs (Bothell, WA)
% Solids, plug 1	0.50 – 0.75 g	20-mL glass scintillation vial	Brooks Applied Labs (Bothell, WA)
Selenium, plug 2	1.0 - 1.5 g	20-mL glass scintillation vial	Brooks Applied Labs (Bothell, WA)
% Solids, plug 2	0.50 – 0.75 g	20-mL glass scintillation vial	Brooks Applied Labs (Bothell, WA)
Selenium, plug 3	1.0 - 1.5 g	20-mL glass scintillation vial	Brooks Applied Labs (Bothell, WA)
% Solids, plug 3	0.50 – 0.75 g	20-mL glass scintillation vial	Brooks Applied Labs (Bothell, WA)
Selenium, plug 4	1.0 - 1.5 g	20-mL glass scintillation vial	Brooks Applied Labs (Bothell, WA)
% Solids, plug 4	0.50 – 0.75 g	20-mL glass scintillation vial	Brooks Applied Labs (Bothell, WA)
Selenium and Percent Solids, fillet 1	20 - 25 g	50-mL HDPE straight-sided jar, or conical HDPE tube with snap top	Brooks Applied Labs (Bothell, WA)
Selenium and Percent Solids, fillet 2	20 - 25 g	50-mL HDPE straight-sided jar, or conical HDPE tube with snap top	Brooks Applied Labs (Bothell, WA)

Analysis	Target Mass	Container Type	Destination
Selenium and Percent Solids, fillet 3	20 - 25 g	50-mL HDPE straight-sided jar, or conical HDPE tube with snap top	Brooks Applied Labs (Bothell, WA)
Selenium and Percent Solids, fillet 4	20 - 25 g	50-mL HDPE straight-sided jar, or conical HDPE tube with snap top	Brooks Applied Labs (Bothell, WA)
Lipid, fish 1	30 - 35 g	Container provided by the analytical laboratory	TBD
Lipid, fish 2	10 - 15 g	Container provided by the analytical laboratory	TBD
Lipid, fish 3	10 - 15 g	Container provided by the analytical laboratory	TBD
Lipid, fish 4	10 - 15 g	Container provided by the analytical laboratory	TBD
Lipid, fish 5	10 - 15 g	Container provided by the analytical laboratory	TBD
Small Archive 1	50 - 60 g	125-mL straight-sided amber or clear glass jar	CSRA Sample Repository (Baltimore, MD)
Small Archive 2	50 - 60 g	125-mL straight-sided amber or clear glass jar	CSRA Sample Repository (Baltimore, MD)
Bulk Archive 1	200 - 220 g	500-mL straight-sided amber or clear glass jar with foil-lined lid	CSRA Sample Repository (Baltimore, MD)
Bulk Archive 2	All remaining mass up to 220 g	500-mL straight-sided amber or clear glass jar with foil-lined lid	CSRA Sample Repository (Baltimore, MD)
Total (to the nearest gram)*	656 - 764 g		

* In the event that insufficient fish tissue mass exists to prepare the required number of aliquots, contact the EPA Project Manager for instructions, per Step 23.

27. Prepare the plug samples and homogenized sample aliquots for **selenium and percent solids**. Weigh an appropriate clean sample container (Table 1) to the nearest 0.5 g and record the weight. Transfer sufficient fillet plug tissue or homogenized fillet aliquots to the container to achieve the target mass for that container in Table 1, weigh the container again, record the weight, and determine the weight of the aliquot to the nearest 0.5 g by difference.

    Note: The archive sample jars are not filled until after sufficient volume for lipids determination has been collected, as described in Step 29. For the sample used for homogeneity testing, the archive jars are not filled until triple the lipid mass is collected (see Step 36).

    When filling jars, leave sufficient space at the top of each jar before sealing with the designated lid to allow for expansion of the tissue as it freezes. *In no case should jars be filled beyond 80% capacity, as this may result in breakage on freezing.* Wipe off the outside of the jars to remove any tissue residue or moisture. Fill out a label for each container using a waterproof marker. Include the following information (at a minimum) on each label:

    - sample identification number,
    - tissue sample type (e.g., fillet plug or homogenized fillet)
    - analysis type (e.g., selenium),
    - plug or aliquot weight (to the nearest 0.5 gram),

- preparation batch ID (assigned by EPA as a numerical sequence from 7 to 18), and
- preparation date (e.g., mm/dd/yyyy)

Affix the label to the container with clear wide tape. Place each container inside one heavy-weight food-grade self-sealing plastic freezer bag to avoid sample loss due to breakage. Freeze the tissue samples at -20 °C, and maintain samples in the freezer until directed by the EPA Project Manager to ship them to the analytical laboratories. (The EPA Project Manager will not issue these instructions until equipment rinsate and homogeneity tests described in Steps 30 to 37 have been completed, reported, evaluated, and determined to be acceptable.)

28. After filling all of the containers with the tissue samples for selenium and percent solids, remove 30 to 35 g of homogenized fillet tissue from fish 1 in the batch (for triplicate lipid analysis) and 10 to 15 g of homogenized fillet tissue from fish samples 2-5 (for single lipid analysis) to be used to determine the lipid content of each sample fillet. Place these aliquots in clean glass or plastic containers of suitable size (provided by the designated analytical laboratory) and label each of them with the sample ID number. Store the lipid aliquots in the freezer at -20°C until they are ready to be shipped to the designated analytical laboratory to perform the lipid determinations in Steps 32, 36, and 37.

29. The archive sample jars are not filled until after sufficient volume for determining lipids has been collected. Once the aliquots for mercury and lipids have been collected, the remaining tissue mass is used to create the four archive samples. Begin by transferring 50 - 60 g of tissue to the first small archive sample container. Continue by transferring a 50 - 60 g aliquot to the remaining small archive container. Ideally, sufficient homogenized fillet tissue mass will remain to produce two bulk archive containers. Therefore, transfer 200 - 220 g of tissue to the first bulk archive sample container. Continue by transferring 200 - 220 g of tissue to the second bulk archive container. However, if less than 220 g of tissue is available, transfer all of the remaining homogenized tissue to the bulk archive container. Seal and label the containers as described in Step 27 for the other aliquots.

    Note: Step 23 requires that the laboratory contact the EPA Project Manager whenever a sample does not yield at least 656 g of tissue. The EPA Project Manager will provide direction to the laboratory regarding samples yielding less than 656 g of tissue that must be followed at this point in the procedure.

    Any tissue that remains after filling the second bulk archive jar may be discarded.

II.E. Equipment Cleaning between Composite Samples

30. All of the homogenization equipment must be thoroughly cleaned between each individual fish sample. Once both of the fillets from the individual sample have been homogenized, disassemble the homogenization equipment (i.e., blender, grinder, or other device) and thoroughly **clean all surfaces and parts** that contact the sample. Similarly, **clean all knives, cutting boards, and other utensils used**. At a minimum:

    - Wash with a detergent solution (phosphate- and scent-free) and warm tap water
    - Rinse three times with warm tap water
    - Rinse three times with deionized (DI) water
    - Rinse with acetone
    - Rinse three times with DI water
    - Rinse with (not soak in) 5% nitric acid
    - Rinse three times with DI water

- Allow the components to air dry

31. Reassemble the homogenization equipment and proceed with homogenization of the next fish sample in the batch (e.g., begin with Step 6 above).

II.F. Lipid Determination for Every Homogenized Fillet Sample

The procedures for determining the lipid content of every homogenized fillet sample from fish 2-5 in a fish sample preparation batch are described in Step 32 below. Additional lipid determinations are required for fish 1 in every fish sample preparation batch, as described in Steps 36 and 37.

32. For fish 2-5 in each fish sample preparation batch, use the 10 to 15 g aliquot of homogenized tissue collected in Step 28 to determine the lipid content of the sample. The laboratory under contract to Tetra Tech (TBD) will extract the aliquot using SW-846 Method 9071B to determine the lipid content of that aliquot, which is recorded in units of percent (i.e., grams of lipid per gram of tissue x 100).

II.G. Quality Control (QC) Procedures

The project-specific QC procedures include preparation and testing of equipment rinsate samples and homogeneity testing, using lipids as a surrogate.

During the sample preparation efforts, the fish sample preparation laboratory will prepare one set of selenium rinsate samples and one homogenized fillet tissue aliquot for triplicate lipid determinations from one of every five fish samples represented in each fish sample preparation batch, as described in Steps 33 to 36 below. The batch-specific rinsate and homogeneity results will be reviewed by Tetra Tech and CSRA. The fish sample preparation laboratory may continue to process up to 3 additional batches during the QC sample analysis and review process. However, the fish sample preparation laboratory may **not** continue beyond that series of 4 batches (initial batch and 3 additional batches) of samples until receiving notification from the EPA Project Manager that review of the initial batch rinsate and homogeneity test results is complete and the results were deemed satisfactory.

Continued sample processing is dependent on both the quality of the fish sample preparation laboratory's efforts and on the timeliness of their delivery of QC results.

Rinsate and Blank Sample Production

33. Once per batch (of 5 fish) during the fish sample preparation operations, prepare a set of rinsate samples prior to reassembling the homogenization equipment (Step 31), as follows:

    - Prepare a **DI water rinsate** using 250-mL of DI water. Collect the DI water rinsate in a clean glass or HDPE container.
    - Place a second aliquot of DI water in a separate similar clean container for use as a blank.
    - Acidify these two samples to pH < 2 with nitric acid. This set of rinsate and blank samples will be analyzed for selenium as described in Step 35.

34. Label each container as either "rinsate -- DI water" or "blank -- DI water," and include the date it was prepared (mm/dd/yyyy), the analysis type (Se), and the preparation batch identifier. Store the rinsates and blanks in a refrigerator at a temperature <6 °C.

Rinsate Analyses

35. During the fish sample preparation operations, a laboratory under contract to Tetra Tech (to be determined) will analyze one set of DI water rinsate and blank samples per fish sample preparation batch for selenium using Inductively Coupled Plasma-Mass Spectrometry as detailed in EPA Method 200.8, Revision 5.4.

Corrective Actions for Rinsates

The rinsate results will be evaluated based on the mass of selenium detected, and assuming that all of the apparent contamination could be transferred to a nominal 50-g mass of homogenized tissue. Results for selenium above the anticipated reporting limits for this analyte in homogenized fillet tissue samples may be cause for corrective actions by the fish sample preparation laboratory. These corrective actions may include revisions to the fish sample preparation laboratory's equipment cleaning procedures, followed by a successful demonstration of the revised cleaning procedures through preparation and analysis of additional rinsate samples.

Lipid Determination to Confirm Homogeneity

36. For fish 1 in each fish sample preparation batch, a laboratory under contract to Tetra Tech will use the 30 - 35 g aliquot of homogenized fillet tissue to conduct triplicate analyses of the lipid content of homogenized fillet tissue samples to confirm that they are homogeneous.

    Remove 30 to 35 g of fillet tissue before filling the archive sample containers. Place this aliquot in a glass or plastic container of suitable size and label it with the sample ID number. Transfer the lipid aliquot to the laboratory under contract to Tetra Tech for triplicate lipid determination. This laboratory will use 5 to 10 g aliquots of fillet tissue for each of the 3 lipid analyses.

37. From the lipid results, calculate the mean lipid content (in percent), the standard deviation (SD), and the relative standard deviation (RSD) using the formulae below, or the corresponding functions in Excel.

$$\text{mean \%lipids} = \frac{\sum_{i=1}^{3} (\%\,\text{lipids})_i}{3}$$

$$\text{SD} = \sqrt{\frac{\sum_{i=1}^{3} (\%\,\text{lipids}_i - \text{mean lipids})^2}{2}}$$

$$\text{RSD} = \frac{\text{SD}}{\text{mean}}$$

If the RSD of the triplicate results is less than or equal to 15%, then the homogenization effort is judged to be sufficient for all samples in that preparation batch. For this sample analyzed in triplicate, the mean lipid content will be the value reported for that sample, following the requirements described in Step 32.

Corrective Actions for Homogeneity

If the RSD is greater than 15%, then corrective action is required for all samples in that preparation batch. Corrective actions will be determined by EPA in direct consultation with the laboratory and Tetra Tech, but the default corrective action consists of regrinding all of the aliquots from each whole fish sample in the affected batch until the RSD criterion is met.

This may entail retrieving all sample aliquots (see Table 1) from the freezer, allowing them to partially thaw, and homogenizing them again, beginning at Step 21. In these instances, all of the equipment cleaning procedures will be repeated between each whole fish sample, new lipid results will be determined for each fish sample, and a new homogenization QC determination (triplicate lipids on one fish sample per batch) will be performed. New sample containers are required for any rehomogenized samples.

II.H. Reporting Requirements

38. The sample preparation laboratory will prepare a weekly progress report to document the status of fish preparation activities and forward the report electronically to EPA. The format of the weekly progress report will be as an Excel spreadsheet using the 2015 Great Lakes Human Health Fish Fillet Tissue Sample fish sample preparation as a guide for organization of the spreadsheet. For each homogenized sample processed or plug sample collected during that period, include at least the following information in the report:

- sample identification number,
- common name for the fish species (provided to the laboratory in the instructions from EPA),
- field-determined length and lab-determined weight of each fish sample,
- total whole fillet (unhomogenized) weight (to the nearest gram),
- homogenized fillet sample (i.e., homogenate) weight (to the nearest gram),
- total laboratory-extracted plug sample weight (to the nearest 0.1 gram),
- analysis type (e.g., selenium, percent solids, lipid, and archive samples),
- aliquot weight (to the nearest 0.5 gram),
- preparation batch ID,
- preparation date (e.g., mm/dd/yyyy),
- QC sample identifiers associated with the batch of homogenized fillet samples, and
- lipid results for each fish sample

The weekly report will be due by COB Monday (or one day later in the case of holidays) and it will document sample preparation progress for the previous week.

In addition, the laboratory must report the results of the rinsate analyses for selenium and the triplicate lipid results associated with the sample batch. Those results **must** be reported to EPA as soon after the analyses as practical to facilitate timely review and to minimize delays in receiving approval to process future batches.

Note: As specified in the QC section of this QAPP, the fish sample preparation laboratory may **not** continue beyond the series of 4 fish sample preparation batches until receiving notification from EPA that review of the initial (in the series of 4 batches) batch rinsate and homogeneity test results is complete and the results were deemed satisfactory.

II.I. Shipping Samples

39. **No samples may be shipped until the EPA Project Manager has reviewed the sample homogeneity testing and rinsate results.** The EPA Project Manager will notify the fish sample preparation laboratory by email when specific samples may be shipped, and to whom.

    Samples are shipped in batches (one batch per cooler) to Brooks Applied Labs. Each fish sample preparation batch produces two selenium analysis batches. One selenium analysis batch contains 20 lab-extracted plug samples for selenium analysis and 20 lab-extracted plugs for percent solids. The other selenium batch contains 20 homogenized fillet tissue samples with sufficient tissue for both selenium and percent solids analyses.

    When shipping batches of pre-frozen fillet tissue aliquots, keep the individual containers bagged in the food-grade plastic freezer bags. Place these bags in a cooler with adequate space for the tissue containers, packing materials, and dry ice. Shipments of paired plug samples for selenium and percent solids analyses may use other forms of packing materials (e.g., bubble-wrap bags, foam blocks with pre-drilled holes) appropriate for the scintillation vials and require larger coolers for shipment of the 40 plug vials than coolers currently in use for shipping 20 plug vials at one time.

    Secure each of the tissue containers with packing materials (e.g., bubble wrap or foam) before adding the dry ice. Place a layer of bubble wrap and a plastic cooler liner on top of the containers before adding the dry ice, as this can prevent cracking the lids.

    The amount of dry ice required for shipping will depend on the number of homogenized fillet tissue samples and lab-extracted plug samples in the cooler and the time of year. It should be an adequate supply to keep the tissue samples frozen for 48 hours (i.e., a minimum of 30 pounds of dry ice per cooler for up to 10 pounds of fillet tissue samples).

    Record the samples contained in the cooler on a shipping form provided by CSRA and place the form in a plastic bag taped to the inside lid of the cooler. Secure the outside of the cooler with sealing tape, address it to the sample recipient identified by the EPA Project Manager, and attach a dry ice (dangerous goods) label. Ship the cooler via an overnight express carrier on a date that will allow delivery of the cooler to the analytical laboratory on a normal business day (e.g., **no Saturday deliveries and no deliveries on U.S. Federal holidays**). Provide the air bill number for each analytical laboratory shipment to CSRA and the EPA Project Manager via email on the day that the shipment occurs. **CSRA will provide the fish sample preparation laboratory with a third-party FedEx account to which each shipment will be billed.**

ATTACHMENT 1
ANALYSES OF RINSATES AND BLANKS FOR SELENIUM

This attachment describes the analyses of rinsate samples and blanks generated during the fish sample preparation process for the selenium phase of the Fish Plug Evaluation Study. The results of those analyses are important in demonstrating that the sample preparation laboratory's equipment cleaning procedures are effective at preventing cross-contamination between fish tissue samples.

A. EQUIPMENT AND MATERIALS:

- Inductively coupled plasma - mass spectrometer (ICP/MS) instrument compatible with EPA Method 200.8 (must be capable of achieving an MDL of approximately 8 µg/L or less).
- Assorted glassware, syringes, etc.

B. RINSATE AND BLANK ANALYSES

Each set of rinsate samples will include:

- One deionized water (DI) rinsate sample and one DI water blank sample for analysis of total selenium.

During sample preparation efforts, the laboratory will prepare selenium rinsates at a frequency of one set for each batch of 5 fish samples prepared. Based on 30 whole fish samples, six sets of rinsates are anticipated.

The analytical laboratory (to be determined) will digest and analyze the selenium rinsates and blanks by Method 200.8, Revision 5.4 (1994) (*Direct analysis of the rinsates and blanks without digestion is **not** permitted.*) For each paired analysis, the laboratory will report the concentration of total selenium in the rinsate and the blank samples.

The analytical laboratory will perform a method detection limit (MDL) study for selenium in aqueous samples, or use existing aqueous MDL data for the ICP/MS instrument employed. The laboratory must be able to achieve an MDL of approximately 8 µg/L or less. Selenium results will be reported down to the MDL for aqueous samples.

C. QUALITY CONTROL

The quality control (QC) procedures required for the rinsate analyses for selenium include:

- MDL study, as described above
- Instrument calibration (see Method 200.8, Revision 5.4 for procedures and acceptance criteria)
- Instrument blanks
- Calibration verification (once per analysis batch consisting of one set of rinsate samples)
- Laboratory control sample (LCS) once per analysis batch (consisting of one set of rinsate samples)

The matrix for the selenium rinsates is reagent (deionized) water, which should not adversely affect method performance. Therefore, matrix spike samples are **not** required for selenium.

The instrument blanks for selenium take the place of a traditional method blank that would be digested along with environmental samples.

The selenium results will be reviewed by CSRA and EPA as soon as they become available for each fish sample preparation batch, and the laboratory will not be authorized to prepare fish tissue samples beyond a series of 4 fish sample preparation batches until that review is complete and the results are acceptable for the initial batch in each series of 4 fish sample preparation batches.

D. DELIVERABLES

Summary data from the rinsate analyses are to be delivered to EPA in an Excel file. That file must contain the following information, at a minimum:

- Batch ID - assigned by EPA (numerical sequence from 1 to 6)
- Sample ID - as described in the instructions for preparing the rinsates
- Lab sample ID - unique internal identifier used by the laboratory, if any
- Prep date - Date (MM/DD/YYYY) on which the rinsate or solvent blank was prepared
- Analysis type - "Selenium"
- Analysis date - Date (MM/DD/YYYY) on which the rinsate or solvent blank was analyzed
- Analyte name – Selenium (total)
- Selenium concentration in µg/L
- Lab qualifiers - as needed to describe any analytical concerns. A complete list of the qualifiers and their meanings must be included with each data submission (e.g., in a separate tab on the Excel file).
- Reporting limit (i.e., the MDL for this study) for selenium - in the same concentration units used for the selenium results
- Instrument calibration data - Submit as a separate tab in the Excel file. Must include results for the initial calibrations for selenium, as well as any relevant calibration verifications associated with the selenium analyses. Include calibration equations (e.g., regressions) and metrics (e.g., correlation coefficient or calibration factor).

Provide Excel files for the selenium analysis results to the Tetra Tech Project Leader. Raw data supporting selenium analysis (e.g., instrument printouts) must be retained by the laboratory and made available to EPA when requested. If requested, raw data may be submitted in hard copy, or as a PDF file.

Appendix E

Fish Plug Evaluation Study Selenium Phase Fish Sample Preparation Laboratory Bench Sheet

Sample ID		Filleter:	Fish _____
EPA		Tissue Processor:	
Prep Date (MMDDYYYY):			

Sample Type	Species	Fish Length (mm)	Fish Mass (g)	Fillet Mass (g)	Fillet Tissue Recovery (%)	Total Homogenate Mass (g)	Homogenate Tissue Recovery (%)
Fillet							
Notes:							

Sample Jar	Se Plug 1 (2 plugs)	Se Plug 2 (2 plugs)	Se Plug 3 (2 plugs)	Se Plug 4 (2 plugs)	Se Plug 1 % Moisture (1 plug each)	Se Plug 2 % Moisture (1 plug each)	Se Plug 3 % Moisture (1 plug each)	Se Plug 4 % Moisture (1 plug each)
Sample ID	LP1	LP2	LP3	LP4	LP1%M	LP2%M	LP3%M	LP4%M
Target Sample	1.0 - 1.5 g	1.0 - 1.5 g	1.0 - 1.5 g	1.0 - 1.5 g	0.50 - 0.75 g	0.50 - 0.75 g	0.50 - 0.75 g	0.50 - 0.75 g
Sample Mass (g)								

Sample Jar	Homogenized Fillet 1	Homogenized Fillet 2	Homogenized Fillet 3	Homogenized Fillet 4	Triplicate Lipids (Fish 1 from each batch)	Lipids (Fish 2 to 5 from each batch)	Small Archive 1	Small Archive 2	Bulk Archive 1	Bulk Archive 2
Sample ID	HF1	HF2	HF3	HF4						
Target Sample Mass (g)	20 - 25 g	20 - 25 g	20 - 25 g	20 - 25 g	30 - 35 g	10 - 15 g	50 - 60 g	50 - 60 g	200 - 220 g	All remaining mass up to 220 g
Sample Mass (g)										